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THE UNIVERSITY OF ALBERTA

SYNTHETIC STUDIES IN THE AREA OF AMPHOTERICIN B MIMICS

by

JANUSZ WIKTOR DAROSZEWSKI ©

A THESIS

SUBMITED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA
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James A. Plambeck
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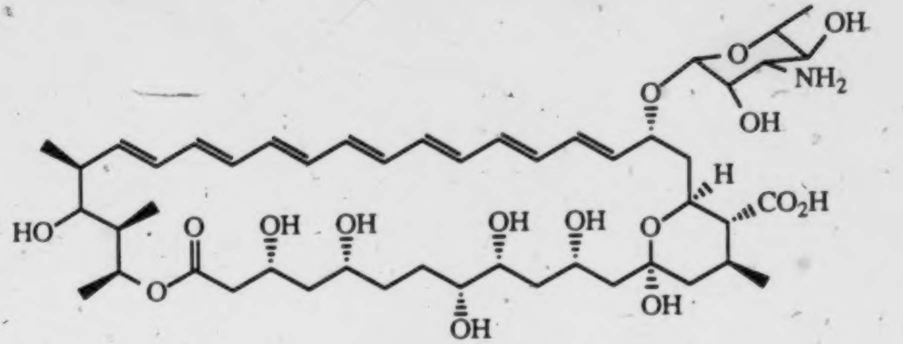
H. C.
....

Date: 21st Aug., 1987

To my Family

ABSTRACT

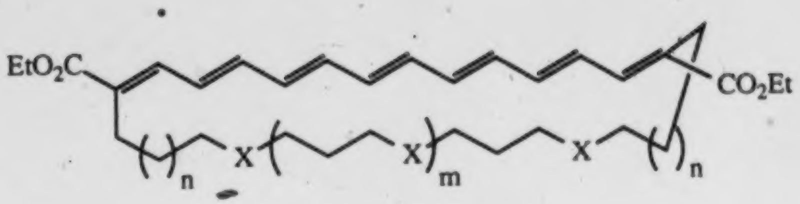
The synthesis of simple analogs of the polyene antibiotic Amphotericin B is described in this thesis.



Amphotericin B

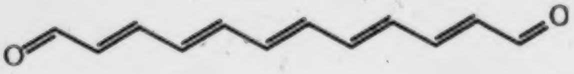
It is believed that the antifungal activity of the polyene antibiotics, and, in particular, their ability to increase cell membrane permeability, is a result of unique structural features characteristic of these materials. Four basic features: the cyclic character, elongated shape, the presence of an extended system of conjugated double bonds, and the presence of a hydrophilic chain were selected and incorporated into the model structures. Six macrocyles of general structure **A** were synthesized.

The syntheses were accomplished by means of Wittig chemistry. Materials **1**, **2** and **4** were obtained by condensation of pentaene dialdehyde **7** with the appropriate diphosphonate **B**.


EtO$_2$C
CO$_2$Et
X
X
X
n
m
n

A

1	n = 1	m = 2	X = O
2	n = 1	m = 2	X = S
3	n = 1	m = 2	X = SO
4	n = 1	m = 1	X = S
5	n = 1	m = 1	X = SO
6	n = 0	m = 3	X = O



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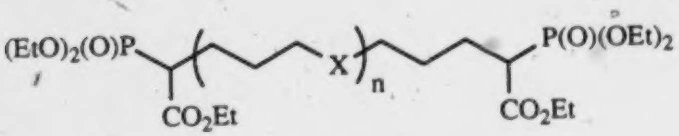
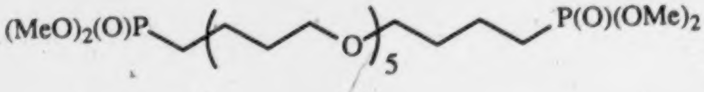

(EtO)$_2$(O)P
P(O)(OEt)$_2$
X
n
CO$_2$Et
CO$_2$Et

B

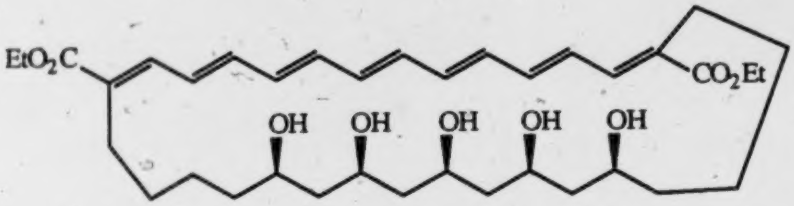
1	n = 4	X = O
2	n = 4	X = S
4	n = 3	X = S

Compounds **3** and **5** were obtained by oxidation of **2** and **4** respectively, with sodium periodate. Compound **6** was prepared by condensation of dialdehyde **7** with the doubly-stabilized reagent obtained *in situ* from diphosphonate **8** and diethyl carbonate.


(MeO)$_2$(O)P
P(O)(OMe)$_2$
O
5

8

Analogs **1–5** were tested for biological activity and were found to be inactive against yeasts. The lack of activity suggested that the hydrophilic chain linking the ends of the polyene should more appropriately be a polyhydroxy unit and the synthesis of compound **9** has been started. The route is based on **7** and the known protected silyl ether **10**.



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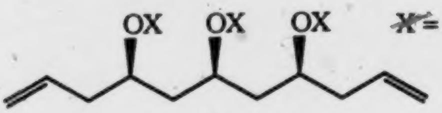

X = *tert*-BuMe$_2$Si-

10

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TABLE OF CONTENTS

PAGE

I. INTRODUCTION 1

- General introduction 1
- Polyene antibiotics 2
- Synthesis of polyenes 20

II. RESULTS AND DISCUSSION 38

- Introduction 38
- Synthesis of heptaenedioic acid **11** 42
- Synthesis of diol **22** 49
- Attempts to esterify diacid **11** and to obtain the macrolactone with diol **22** 54
- Attempts to generate the macrolactone by an alternate process 59
- Use of the Wittig reaction and cyclization of modified model compounds 61
- Attempts to improve the synthesis of macrocycle **70** 67
- Synthesis of sulphur analogs of **70** 79

III. CONCLUSION 95

IV. EXPERIMENTAL 96

REFERENCES 155

APPENDIX A 166

APPENDIX B 190

APPENDIX C 192

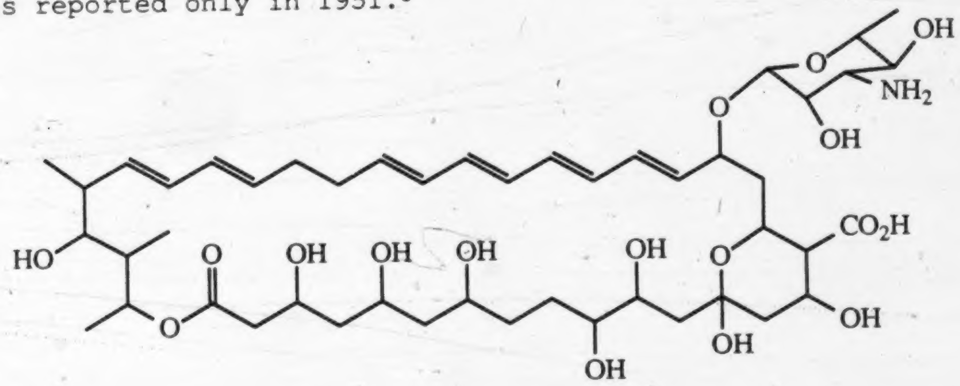
LIST OF FIGURES

FIGURE		PAGE
1.	Complex of Filipin with cholesterol	6
2.	Channel formed by Amphotericin B	8
3.	CPK model of lactone **23**	41
4.	Spatial disposition of hydrogen atoms α and β with respect to carboethoxy group in **70** and **71**	67
5.	X-Ray structure of **102**	90
6.	Molecular model of **102**	91
7.	Molecular model of **105**	92
8.	CPK model of **112**	169

I. Introduction.

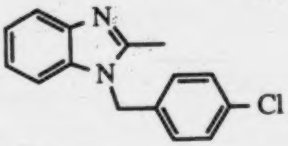
I.1. General introduction.

Fungal infections in man are often difficult to cure and one of the reasons is an acute shortage of effective drugs. Chemotherapy in this area has a rather brief history. The first report of successful treatment of a case of a fungal infection (sporotrichosis) dates back to 1903[1], and the agent used, potassium iodide, was not a very sophisticated one, at least from a chemical point of view. Surprisingly, the compound is still used in certain cases.[2] The first antifungal antibiotic of natural origin, Griseofulvin, a metabolic product of *Penicillium griseofulvum*, was discovered in 1939, and the first therapeutically useful antifungal drug, active against yeasts and yeast-like fungi, Nystatin **1**, was reported only in 1951.[3]



1

The discovery of Nystatin inaugurated a string of reports on similar substances produced by members of the genus *Streptomyces*. All of the compounds were categorized as polyene antibiotics and they constitute a pool of powerful antifungal agents. As far as synthetic antifungal materials are concerned, the most important is a group of chemically related drugs derived from imidazole, such as Chlormidazol **2**, Clotrimazol **3** and Miconazol **4**.



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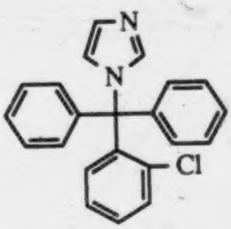

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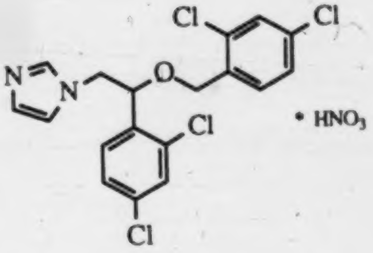

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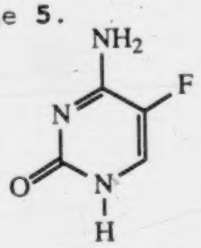
Another example of a synthetic antifungal drug, which almost completes the list of therapeutically useful substances, is Flucytosine **5**.

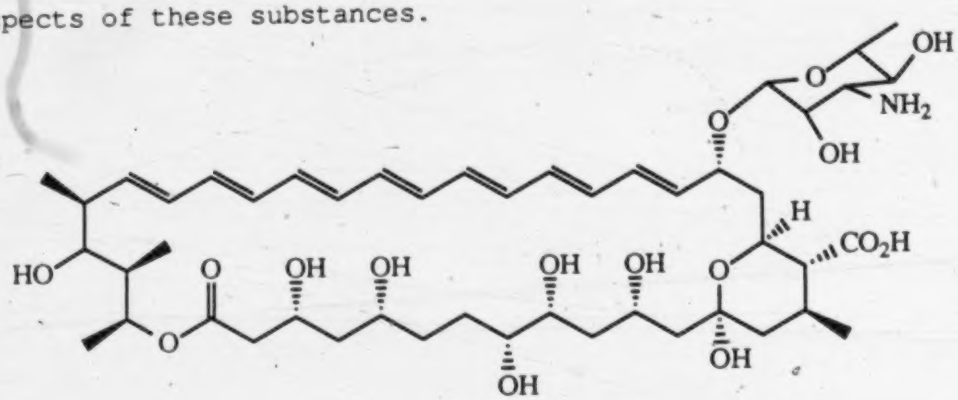


5

Interestingly, compound **5** was actually synthesized with cytostatic activity in mind[4] and found to be inactive, but, fortunately, it was tested later for other types of biological activity and was found active *in vitro* against yeasts. Tests *in vivo* showed that **5** was active in experimental candidosis in mice and, eventually, Flucytosine was recognized as a valuable antifungal material, especially when administered with other drugs.

I.2. Polyene antibiotics.

Polyene antibiotics are the most important antifungal agents and have attracted a lot of interest. Although almost 40 years have passed since the discovery of Nystatin, there still is a considerable amount of uncertainty about many aspects of these substances.



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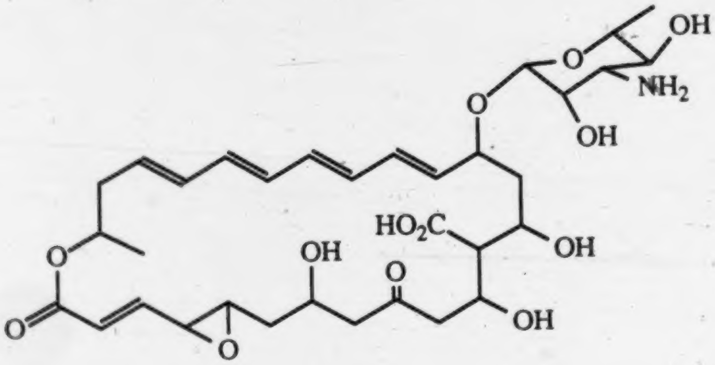
First of all, difficulties in separation and purification led to some confusion. Substances thought to be homogeneous were shown later to be mixtures while substances identified as being different were proven identical.[5] However, the advent of modern separation techniques removed most of the obstacles of this type. Due to difficulties in obtaining pure samples, and because of the intrinsic complexity of polyene antibiotics, the structural determinations are difficult, and although there are some 200 known members of this group, the only one for which the absolute configuration is known is Amphotericin B (**6**) (AmB).[6]

Together with Nystatin and Natamycin **7**, it is one of only a few polyene antibiotics that are used clinically.



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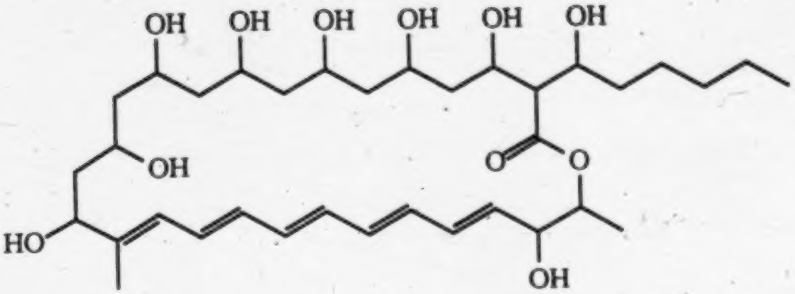
I.2.1. Characteristic features of polyene antibiotics.

Polyene antibiotics as a group are characterized by a few well-defined structural features. First of all, they are cyclic materials with the ring size up to almost 40 atoms. The macrolide is closed by a lactone functionality. There are several conjugated double bonds in the molecule (3–7) and they are confined to the macrolide ring. There are also several hydroxyl groups (6–14), which are usually distributed in a 1,3-fashion around the ring, except for the section containing conjugated double bonds. Finally, although not all the members of the group possess this feature, there is a polar moiety appended to the ring—a carboxyl group, an amino-sugar, or both.

I.2.2. Mode of action of polyene antibiotics.

Since the discovery of the antifungal activity of polyene antibiotics, much effort has been directed towards elucidation of their mode of action, and several techniques had been used to this end. There are a number of detailed reviews covering this area[1,7-9] and other topics related to these substances. The early findings indicated that the site of action of polyene antibiotics is the fungal cell membrane, and that the net result of their action is an increase in the permeability of the membrane. This leads to disturbances in normal functioning of the cell and eventually to its demise. Numerous experiments laid the foundations for the mechanism of action, which was suggested in 1974,[10,11] and with certain refinements, is still accepted. The basic features of the proposed mode of action may be summarized as follows: The polyene antibiotics form complexes with steroids present in the membrane. These complexes interfere with the structure of the membrane and cause leakage of ions and other components of the intracellular fluids and ultimately, death of the cell. However, the character of the complexes depends on the polyene in question and is related to its structure.

Filipin **8** is thought to form planar arrays in which the



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hydrophilic parts of all molecules are on one side of the plane, and the hydrophobic part on the other. Two such arrays form a sandwich, illustrated in Fig. 1, through hydrogen bonds between hydroxyl groups. The sterol, (in the case analyzed,[10] cholesterol) is complexed with the hydrophobic exterior of this sandwich forming two additional layers (Fig. 1).

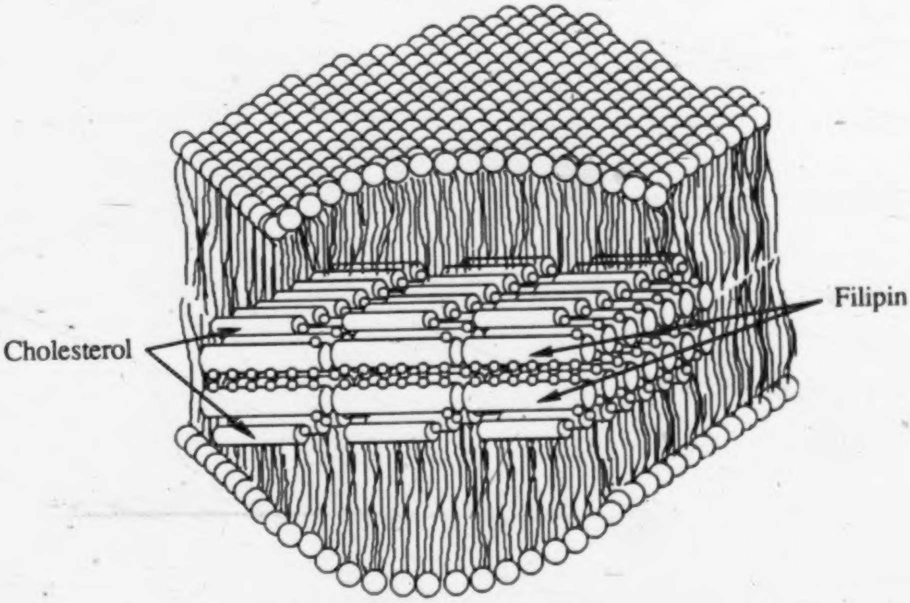


Fig. 1. Complex of Filipin with cholesterol.

The actual mechanism of formation of this type of aggregate is not clear, but it was envisioned that a complex of one molecule of cholesterol and one molecule of filipin may associate with another such pair by hydrogen bonds between molecules of the polyene, thus reducing unfavorable interactions with the hydrophobic interior of the membrane where the whole action takes place. Such an aggregate may be further extended by joining another unit, formed by the same process, to give finally four stacked layers: cholesterol-filipin-filipin-cholesterol.

There is evidence that such structures do exist. For example, freeze-etch electron microscopy revealed[10] the existence of areas of convex deformation (or bubbles) of the membrane surface, indicating the internal presence of Filipin aggregates. These areas projected up to 50Å above the surface. It is believed that curvature at the edges of these deformations induces considerable stress, and that this is the place where rupture of the membrane may occur.

In the case of AmB and Nystatin a different scenario was suggested. Eight molecules of polyene and eight molecules of cholesterol associate in such a way that, by interlacing molecules of both components in a radial fashion, a tubular aggregate is formed. Such an aggregate would have a hydrophobic exterior and a hydrophilic interior and would be of a length equal to half the width of the membrane. When positioned perpendicular to the membrane it would form a half-channel. By bringing two aggregates on either side of membrane together, a pore would be formed, which may be additionally stabilized by hydrogen bonding between hydroxyl groups of the polyene macrolide which are located at its terminus. The other end of the polyene, which in the case of AmB and Nystatin, is very polar, would extend beyond the surface of the membrane and, of course, a free flow of ionic and nonionic species, in and out of the cell would be possible (Fig. 2).

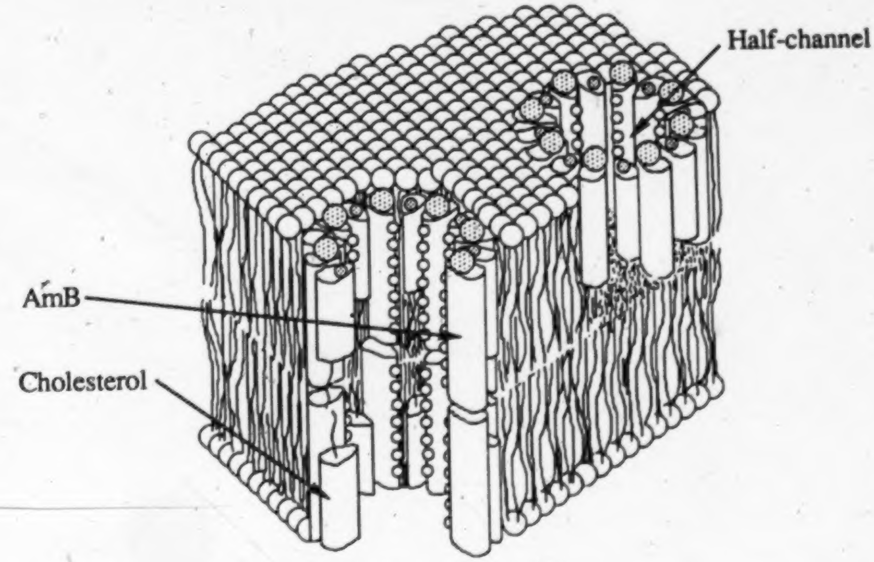


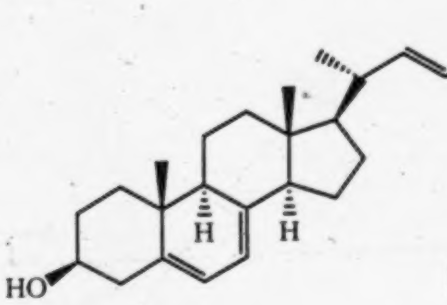
Fig. 2. Channel formed by Amphotericin B.

I.2.3. Amphotericin B.

Amphotericin B is one of most widely used antifungal agents. Because of its relatively low toxicity, it is the only polyene antibiotic which may be used in deeply rooted fungal infections. It is one of the most active drugs available, and a recent study in Japan[12] shows that AmB has *in* *vitro* the lowest minimum inhibitory concentration (MIC) among the drugs used for systematic fungal infections or drugs undergoing clinical tests in Japan, including Ketoconazol, Fluconazol, Itraconazol, Miconazol, Nystatin and Flucytosine. Because of its importance, it has been investigated extensively in many respects, and the results of some of these studies will be discussed.

I.2.3.1. Mode of action of Amphotericin B.

The basic model of interaction between AmB or Nystatin and a cell membrane as described above, is now widely accepted. Additional information collected over the last few years supports the channel hypothesis, although some of the findings are difficult to explain.



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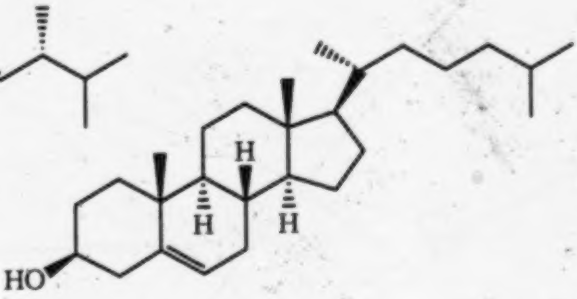

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First of all, the basic tenet of the theory, namely that AmB forms complexes with sterols, has been firmly established. Differential UV spectra were used[13] to show the formation of such complexes in aqueous methanol, ethanol, or propanol. The conditions for the experiments were optimized to show that AmB exhibits a preference for ergosterol **9** over cholesterol **10**.

The mechanism of formation of these complexes is intriguing, as shown by UV studies in propanol.[14] It was found that the rate of complexation depends on the aggregation state of AmB. For monomeric AmB no complexes were formed, but with an increase in concentration of small aggregates (probably dimers), the reaction grew faster and became virtually instantaneous for high concentrations of aggregates. However, the stoichiometry of this complex (AmB:sterol) was found to be 2:1. Since the ratio in the membrane channels is believed to be 1:1, it is not clear how relevant these studies are to the natural situation.

Investigation of the interactions of AmB with sterols in membranes by means of EPR[15-17] showed the existence of 1:1 complexes with cholesterol. Similar results were obtained with the use of Raman spectroscopy.[18] Again, formation of 1:1 complexes with sterols was detected. However, another group of researchers,[19] using ^{14}C labeled AmB and radiometric techniques, as well as measurements of the surface tension of the membrane, reported the formation of complexes with cholesterol and ergosterol in monolayers with a 2:1 ratio. This work also confirmed the fact that complexes with ergosterol are stronger. The last phenomenon has been observed several times and quantitative results show[20] that AmB binds to ergosterol about one order of magnitude more tightly than to cholesterol.

As far as the interactions of AmB with membranes are concerned, absorption and fluorescence spectroscopy have been used to show that AmB binds to membranes. The same technique has been used to estimate the binding constants between AmB and the membrane.[21] It was found as well,[22] that AmB accelerates the transbilayer reorientation of exogenously incorporated lysolecitin. (Another polyene antibiotic, pimaricin, believed not to be able to form channels, was found inactive in this regard.) Additionally, an increased rate of cleavage of the outer layer phosphatidylcholine by phospholipase A_2 was observed, as well as increased accessibility of the inner membrane layer to lipase.

The crucial effect of AmB on the membrane, increased permeability, has been investigated in many systems and by many techniques. Conductance studies showed that, apart from artificial membranes and membranes in human and fungal cells, AmB causes loss of membrane potential and membrane resistance in the plasmalemma of the root hairs of the water plant *T. bogotensis*, with concurrent release of Cl^- and K^+ from the root cell.[23]

EPR was used as well to study the impact of AmB on the membrane permeability.[24] Tempocholine was enclosed in small vesicles suspended in a solution of ascorbic acid, and increased permeability was shown by a rapid signal decay after addition of AmB to the system. The size of the pores was estimated[25] to be ~ 8 Å on the basis of blockage of the flow of ions, as observed by decreasing conductance in the presence of organic molecules of 6–8 Å in size. The selectivity of action of AmB, when compare with Filipin, was evaluated[26] in studies on erythrocytes and liposomes. AmB caused leakage of K^+ without the release of haemoglobin from erythrocytes or ^{14}C labeled dextran from liposomes, whereas Filipin induced nonselective release of solutes in either case. At the same time, it was shown[26] that AmB is about 15 times more active in inducing K^+ leakage from liposomes containing ergosterol than from those containing cholesterol.

However, it seems now that there are two different types of pores formed by AmB. Circular dichroism (CD) studies of interactions of AmB with membranes[27] of human erythrocyte ghosts indicated formation of two types of complexes which preserve their spectral character over the range of concentrations at which AmB induces leakage of K^+. This indicates the existence of only one species of bound AmB for each type of complex. Two different types of pores in membranes containing ergosterol were detected in another study.[28] The small ones leaked urea and salts, while the larger ones were capable of causing the leakage of glucose. CD studies of complexes of AmB[29] with sterols in membranes showed that AmB may assume many conformations, but only two conformers can induce permeability. Judging from the appearance of the measured spectra, the same conformers are involved in complexes with cholesterol and with ergosterol. Additionally, it was found that the concentration of AmB necessary to bring about formation of such complexes is higher in the case of cholesterol. These findings are corroborated by recent computer calculations[30] of the energetics of AmB-cholesterol complexation in the membranes. The calculations show that there are two stable states. The first is stabilized by electrostatic interactions between charged groups of neighboring antibiotic molecules. Another one, with radial orientation of AmB molecules, is stabilized mainly by van der Waals forces, and has a smaller diameter.

Although most of the reported results seem to be in accord with accepted theory, they should be interpreted very cautiously since the conditions of the experiments and the materials used, especially in the case of artificial membranes, may change the behavior of AmB. For example, studies[31] on large unilamellar vesicles (LUV) suggest that LUV may constitute a better model for a cell than small unilamellar vesicles (SUV). When LUV were made with dimyristophosphatidylcholine (saturated acyl chain) a strong, non-saturable binding of AmB to lipid (with the AmB to lipid ratio up to 0.5) was observed along with leakage of carboxyfluorescein. The addition of cholesterol or ergosterol had no influence on the results. However, when egg phosphatidylcholine was used (unsaturated acyl chain), binding was saturated at very low values of the AmB to lipid ratio (0.005), a phenomenon not observed with SUV, and, although leakage of Ca^{++} was detected, there was no leakage of carboxyfluorescein. At the same time, addition of sterols gave rise to different CD spectra for cholesterol and ergosterol. Again, this was not observed with SUV. Some interesting results,[32] which are not easy to interpret, were obtained during investigations on the influence of AmB on small vesicles made with egg phosphatidylcholine. H^+/OH^- permeability was studied by means of potential-dependent paramagnetic probes. In the presence of AmB (1–10 molecules per vesicle) the permeability increased moderately (some 4–8 times) over the background value. Addition of cholesterol however, had a dramatic impact, and the increase in permeability was more than two orders of magnitude. However, ergosterol had no influence at all; in fact, at a 5-15% level of ergosterol in the vesicle membrane, the activity of AmB was even smaller that in the case of the pure egg phosphatidylcholine membrane. Moreover, no significant influence of the presence of sterols on K^+ current was noticed.

To close the issue of permeability, it was suggested[33] that AmB induces leakage of potassium ions from human erythrocytes by a more complex mechanism than just formation of channels. At 5 µM concentration of AmB a total inhibition of the Na^+/K^+ pump was observed, possibly due to interactions with membrane enzymes. Another study,[34] this time on an artificial membrane, showed that addition of a monoclonal antibody of IgM type greatly increased the lifetime of the channel (as shown by measurements of the ionic conductivity of the lipid bilayer) and it was suggested that three antibodies interact with the channel to stabilize its open state. Such a possibility exists in a living cell. There are reports which go even further, challenging the channel mechanism, at least in the case of cation transport.[35] It was shown by NMR studies that Nystatin (which is supposed to act similarly to AmB) associates weakly with sodium ions in methanol (shorter T_1 for ^{23}Na, broadening of sodium signal and changes in ^1H NMR of Nystatin in the region corresponding to the amino-sugar moiety). Quite likely, in the hydrophobic environment of the membrane the interactions may be strong enough to enable a 'shuttle' mechanism for transport of cations through the membrane. Obviously, this model does not explain the increased permeability for anions and neutral solutes. In another study,[36] the kinetics of K^+ release and cytotoxic activity of Nystatin and AmB on *Candida Albicans* has been compared. The results show that Nystatin is more effective than AmB in causing K^+ leakage, whereas the reverse is true for cell mortality. This suggests that the aqueous channels or pores formed by polyene antibiotics are not central to the lethal action of these drugs.

The above data indicate that there are possibly several variations of the basic mechanism by which AmB increases the permeability of membranes, and quite likely, the indiscriminate disruption of the membrane, much as in the case of Filipin, plays a role as well. However, regardless of which mechanism is actually in operation, it is obvious that AmB is capable of inducing increased permeability in natural and artificial membranes.

As far as the relative importance of the functional groups of AmB on its activity is concerned, some information has become available from a recent QSAR study.[37] Data on biological activity (antibiotic activity, erythrocyte K^+ release, hemolysis and some sterol binding properties) of AmB and 16 derivatives (where chemical modifications were made on the sugar moiety or carboxylic acid group) were analyzed by means of principal component analysis. The results indicate that the presence of a positively charged nitrogen atom is important for the biological activity. It was found as well that the lack of a free carboxylic group favors differentiation between the cholesterol and ergosterol (see **9** and **10**) containing cells. ~~However,~~ due to the nature of this type of analysis, it is difficult to draw conclusions which extend beyond the set of analyzed structures.

I.2.3.2. Therapeutic value and toxicity of Amphotericin B.

The fact that AmB forms complexes with steroids raises the question whether there are any structural requirements for the steroid to allow it to engage in the interaction with the polyene antibiotic. It was shown[38-40] that the sterol should posses a 3β-hydroxyl group, that it should have a planar ring system, and a hydrophobic chain at C-17. However, the finding of greatest importance is, as mentioned in the previous section, that AmB has a greater affinity for ergosterol than cholesterol. Actually, this is precisely why

AmB may be used as a drug. Human cell membranes contain mainly cholesterol, whereas fungal membranes are built with ergosterol. This explains as well the lack of antibacterial activity of AmB; bacteria do not have ergosterol in their membranes. Nevertheless, AmB is active, although to a lesser extent, against cells containing cholesterol, and this is the reason behind its low therapeutic index, and is the reason for the high toxicity of all polyene antibiotics.

There are several ways to circumvent the problem. The presence of other substances may influence the selectivity of AmB and it has been found that sucrose monolaurate decreases binding of AmB to cholesterol without affecting binding to ergosterol. Tests on human erythrocytes and cultured mice fibroblasts (L-929) in the presence of this substance, showed that the toxicity of AmB was decreased, whereas the toxicity for *Candida albicans*, under the same conditions, did not change.[41] Another solution to the high toxicity of AmB is the formulation of the drug. In tests on membranes, AmB, when enclosed in vesicles, did cause an increase of the inductance of planar bilayers under conditions precluding exchange of AmB with the membrane. The conclusion was drawn that fusion of vesicles with the membrane had occurred[42] and that this may be a good way to deliver the drug to the site of action. Liposomal AmB was shown to cause less K^+ leakage from human erythrocytes than free AmB. At the same time it was found to be more toxic against *Candida albicans*.[43] A similar finding[44] shows that liposome-bound AmB is inactive toward human erythrocytes and active against promastigotes of *Leishmania* species. In fact, there had actually been a report[45] on tests on mice with liposomal AmB. The drug was encapsulated in small unilamellar vesicles made of egg phosphatidylcholine, cholesterol, and tocopherol succinate in a 9:2:1 molar ratio. The liposomes exhibited good stability in serum and, when used to treat experimental Candida in mice, were most effective when used in doses which were lethal in the case of free AmB.

I.2.3.3. Non antifungal properties of Amphotericin B.

As one can see, AmB is a very potent and useful antifungal agent, but as many studies have shown, its potential is far greater. A few areas which have stirred most interest in recent years will be presented briefly.

AmB was shown to potentiate the immunological response, and although the mechanism of this action is not clear, there are many examples. In a study on mice, AmB was found to activate macrophages against fungi.[46] In a similar study, AmB was found to increase the resistance of mice against *Candida albicans* when administered before infection.[47] At the same time, increased resistance to *Staphylococcus aureus* was observed as well. The appearance of a highly candidacidal cell population in spleen, with properties of activated macrophages reactive against yeasts *in vitro*, was detected. Normal macrophages from mice were activated *in vitro* with AmB to become cytotoxic against *Candida albicans*. Very promising results were obtained[48] on a rat model of progressive pulmonary aspergillosis, a major threat for transplant recipients and patients receiving cancer chemotherapy. AmB, administered as an aerosol before the infection drastically increased the survival ratio. It was found effective as well when administered after infection, although to a lesser extent.

It was demonstrated *in vitro* that AmB is capable of circumventing and reversing the drug resistance of tumor cells, and it is believed that the effect is due to changes in cell membrane permeability.[49] There have been a large number of publications dealing with the AmB-induced enhancement of cytotoxicity by anticancer drugs. These works are covered in a recent review.[50] Finally, in an area which recently seems to be the main challenge for researchers involved in development of new drugs, AmB methyl ester has shown promising activity *in vitro* against the AIDS virus.[51]

The last example of the potential versatility of AmB is disclosed in a patent issued for the use of AmB as a preventive measure against coronary diseases. Studies on rats showed that administration of AmB facilitated the removal of tissue cholesterol by improving its binding to high density lipoproteins.[52]

I.3. Synthesis of polyenes.

In many natural products an important part of the chemical structure is the system of conjugated double bonds. Polyene antibiotics, as shown above, comprise one group of such substances. Another group of great importance is carotenoids and related materials, and there are numerous examples of polyene moieties in many other products found in nature. However, as far as the synthesis of polyene systems is concerned, carotenoids have received the most attention, and this area furnishes most of the literature examples.

Analysis of the strategies employed in building a system of conjugated double bonds shows that basically three types of processes are used. The first type is the introduction of a new double bond to an existing system, without altering the carbon skeleton. Reactions most often used to achieve this are: β-elimination reactions such as dehydration of alcohols or dehydrohalogenation of halides, selective hydrogenation of a triple bond to a double bond, isomerization of a system containing a triple bond and a single bond to a system containing two double bonds, etc.

The second type of process involved, does not produce a double bond directly, but instead sets the stage for a process of the first type, and is usually accompanied by the extension of the carbon skeleton. Examples are: allylic bromination, aldol condensation, reaction of organometallic species with carbonyl compounds, condensation of vinyl ethers with acetals, and coupling of terminal alkynes.

The third type is a combination of the previous two: the carbon skeleton is extended with the concomitant formation of a double bond. Examples of the reactions involved are: Wittig olefination, Peterson reaction, and McMurry coupling. This division is admittedly a simplification of the real picture as aldol condensation is often accompanied by dehydration, the Wittig reaction may be stopped at the the betaine stage, and indeed there are modifications (e.g. Schlosser reaction) which actually rely upon this, β-hydroxy silanes in the Peterson reaction are usually isolated before olefination, and the McMurry coupling may be viewed as a sequence of a pinacol coupling followed by conversion of the 1,2-diol to a olefin.[a]

There are only a few reactions which lead to formation of more than one double bond at the same time. Therefore, a synthesis of a polyene system either comprises several steps whereby double bonds are added to the existing system one at a time, or some type of synthons are first assembled and then coupled to give more a complex structure. Because of this, it is difficult to classify a particular synthesis according to the type of reaction used. Quite often, at different stages, different strategies are employed. It is interesting to note, however, that most of the following examples rely on substrates possessing a carbonyl group, and it seems that this functionality is pivotal to most syntheses of polyunsaturated materials.

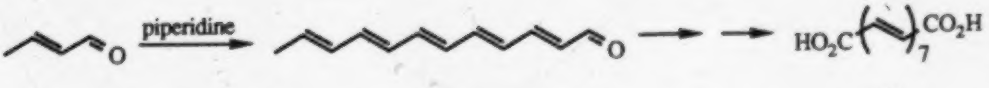
---

[a] The composition of the reaction mixture, that is, the ratio of 1,2-diol to olefin, depends on the reaction conditions.

As already mentioned, there are numerous reports in the chemical literature on polyene synthesis, mainly in the field of synthesis of carotenoids. It is beyond the scope of this introduction to compile a comprehensive review; rather, some representative examples will be presented in more or less chronological order. The issue of the geometry of double bonds will be treated only briefly with the main emphasis placed on all-trans systems.

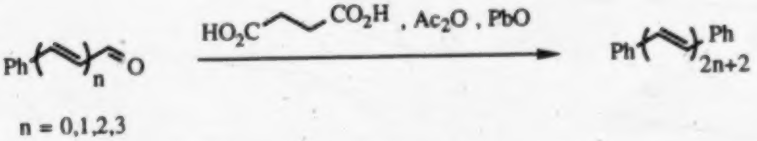
An early example of the synthesis of substances possessing a conjugated system of double bonds was based on the aldol condensation.[53] Under the conditions employed, the extension of the carbon skeleton and formation of new double bonds occurred at the same stage. Moreover, the newly formed material was involved in yet another condensation that resulted in the formation of a mixture of products and low yields. However, the procedure was successfully used to obtain a series of polyunsaturated acids and their derivatives, for example diacid **11** (Scheme 1).[53]

Scheme 1



11

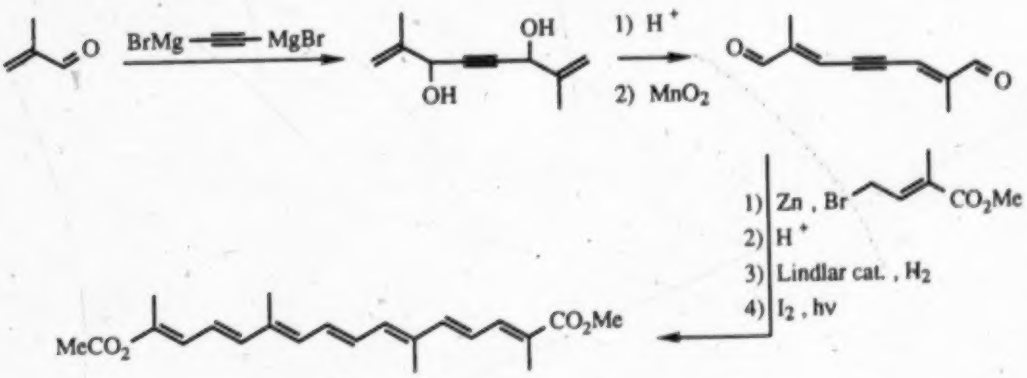
Aldehydes obtained in a similar fashion, when subjected to aldol condensation with succinic acid followed by dehydration and decarbonylation, afforded a series of polyene hydrocarbons (Eq. 1).[54]



Eq. 1

An important improvement over the previous approach was made when the aldol condensation was replaced by the reaction of an organometallic substrate with a carbonyl component. For example, a terminal acetylene was converted to a Grignard reagent or metallated with sodium or lithium, and condensed with an aldehyde or ketone. Now, the construction of the carbon skeleton occurred first and was followed by formation of the double bond. To this end, several approaches have been used. Most often, selective hydrogenation of a triple bond to a double bond in the presence of Lindlar[55] catalyst was employed. This process leads to a cis isomer (other, highly selective catalysts are available now[56]) and, as an all-trans system was usually of interest, an isomerization was carried out with the use of light (sometimes in the presence of iodine). Later on, a milder procedure employing only reflux in low boiling solvents in the presence of catalytic amounts of iodine was adopted. Another example of the use of organometallic chemistry in this type of synthesis is the Reformatsky reaction. Again, the assembly of the carbon skeleton was usually followed by formation of a double bond through dehydration, and the choice of Reformatsky reaction allowed one to incorporate in advance the ester functionality required in the final product. Most of the above is exemplified in the synthesis of the dimethyl ester of Crocetin **12**,[57] a diacid whose derivatives occur in varieties of the gardenia and crocus family, and especially in safron (Scheme 2):

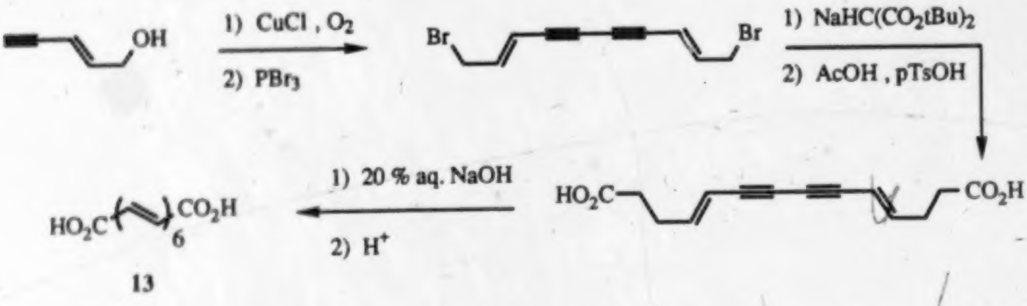
Scheme 2



12

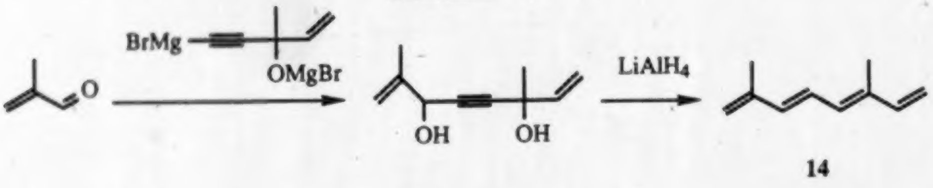
As acetylenes proved to be very attractive substrates, several other techniques for conversion of alkynes to alkenes were devised. One of these involves isomerization of a large part of the carbon chain in such a way that a single bond and a triple bond are converted into two double bonds. In this way, Corticrocin **13**, a bright yellow pigment found in the roots of Norway spruce and Scots pine, was synthesized[58] (Scheme 3).

Scheme 3


OH
1) CuCl, O₂
2) PBr₃
Br
Br
1) NaHC(CO₂tBu)₂
2) AcOH, pTsOH
1) 20 % aq. NaOH
2) H⁺
HO₂C
CO₂H
HO₂C
CO₂H
6
13

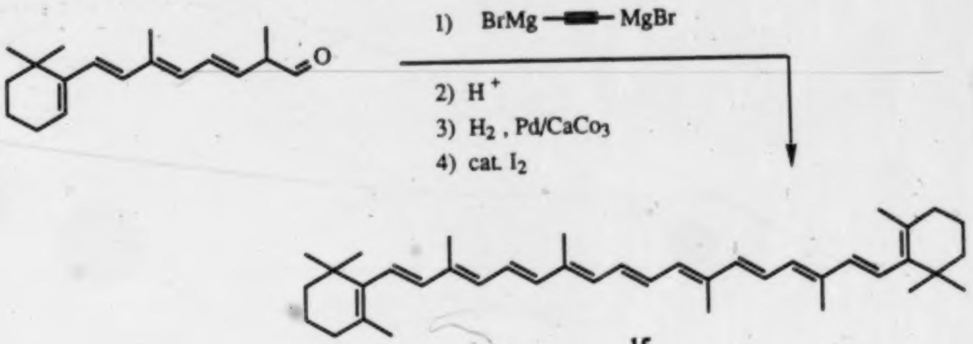
Yet another possibility for converting an alkyne into an alkene is the reduction of an acetylenic diol (formed, for example, by reaction of a dimetallated acetylene with two molecules of a carbonyl compound) with lithium aluminum hydride. This reduction was used originally in a slightly different situation to prepare Cosmene **14**, a naturally occurring hydrocarbon[59] (Scheme 4).

Scheme 4


O
BrMg
OMgBr
OH
OH
LiAlH₄
14

Obviously, condensation of organometallic substrates with carbonyl compounds offers a lot of flexibility in the choice of reagents, and when bifunctional substrates are used (such as a dicarbonyl compound or dimetallated acetylene), a facile assembly is possible of a large symmetrical molecule such as β-carotene **15**, as shown in Scheme 5.[60]

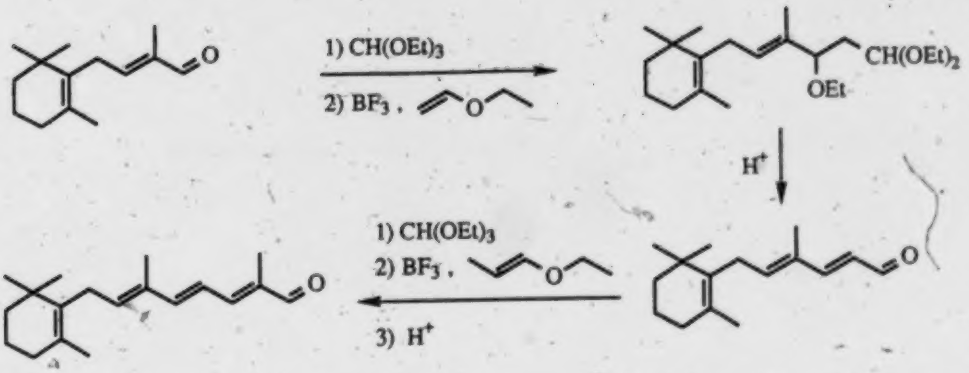
Scheme 5



15

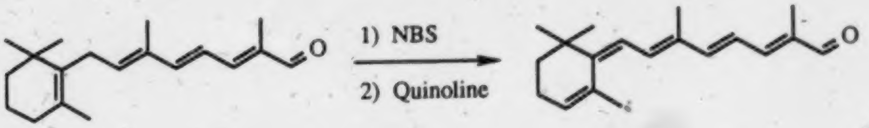
Another reaction, which has been used to synthesize polyenes in an iterative fashion, is that between acetals and vinyl ethers catalyzed by boron trifluoride. In the first step, the carbon chain is extended. Next, acidic workup of the product affords an unsaturated aldehyde, which

Scheme 6



may be utilized in a number of ways, notably, it can be ketalized and subjected again to the above procedure (Scheme 6).[61]

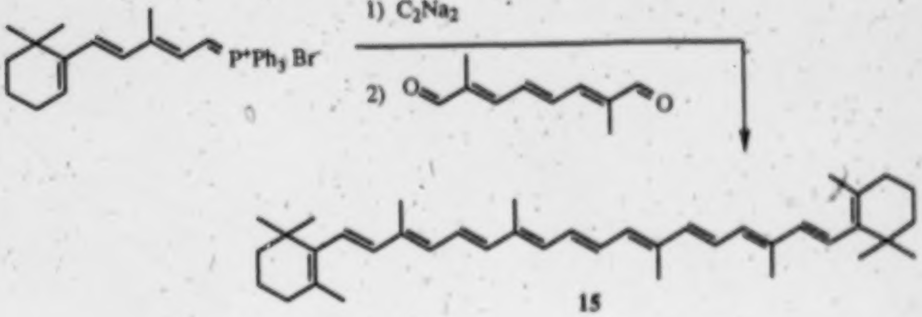
Allylic bromination and elimination has also been used in the synthesis of polyenes. Each of the above aldehydes was converted to the corresponding fully conjugated system[62] as shown in Eq. 2.



Eq. 2

When Wittig published his paper[63] in 1953 on what is now known as the Wittig reaction, a new era in the synthesis of alkenes began. Since then, this reaction has been used extensively and has been modified in many ways to offer greater flexibility in terms of the reactivity of the reagents and the stereocontrol of the reaction.[64]

Scheme 7

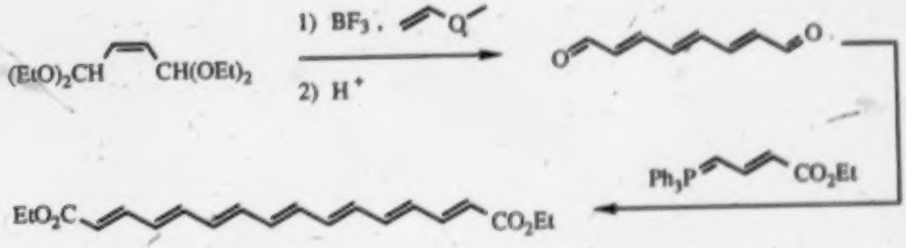

P⁺Ph₃ Br⁻
1) C₂Na₂
2)
O
O

15

One of many examples of the application of this reaction is the synthesis of β-carotene **15** shown in Scheme 7. This process has been patented by Wittig.[65]

A combination of coupling of acetals and vinyl ethers with Wittig chemistry was used to obtain a series of diesters of conjugated polyunsaturated diacids.[66] One example, leading to diester **16**, is shown in Scheme 8.

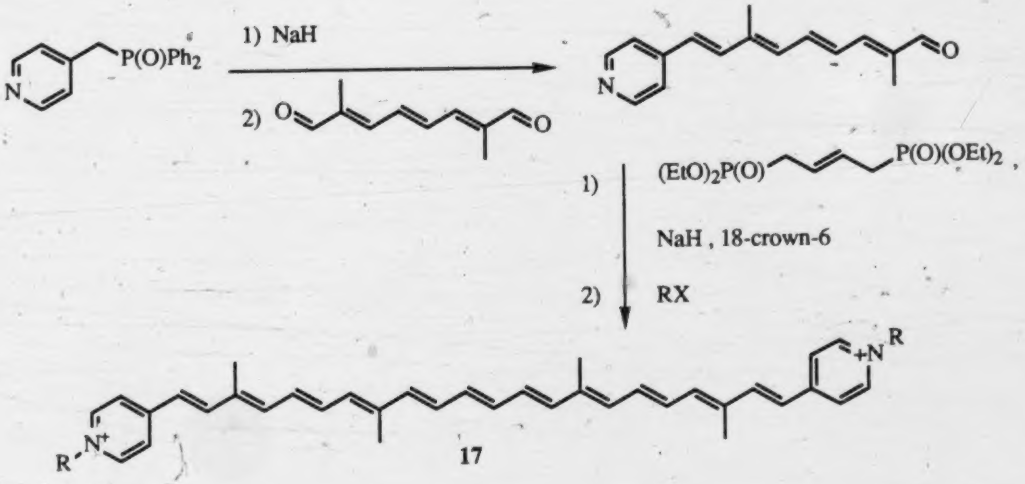
Scheme 8


(EtO)₂CH
CH(OEt)₂
1) BF₃ .
O
2) H⁺
O
O
Ph₃P
CO₂Et
EtO₂C
CO₂Et

16

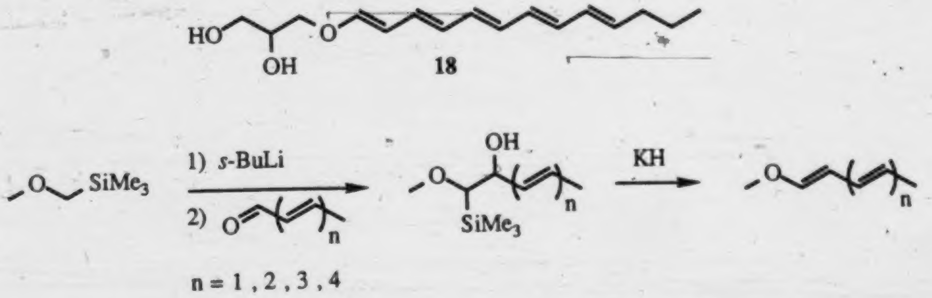
Another example of the use of the Wittig reaction to assemble an extended conjugated system of double bonds is the synthesis of a substance which may be capable of performing the function of a 'molecular wire'. Such a material, when incorporated in the membrane, may act as an electron channel and could serve a variety of purposes in, for example, redox reactions across the membrane. A model substance **17** was synthesized[67] in a concise manner by two consecutive Wittig reactions (Scheme 9).

Scheme 9

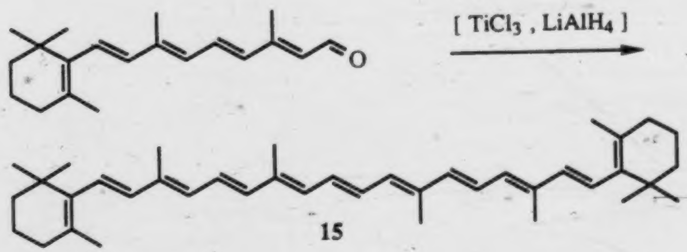


The silicon equivalent of the Wittig reaction, i.e. the Peterson elimination, was used to prepare analogs of fecapentaenes, [for example fecapentaene-12 (**18**)] substances isolated from human feces and suspected of causing colorectal cancer (Scheme 10).[68]

Scheme 10



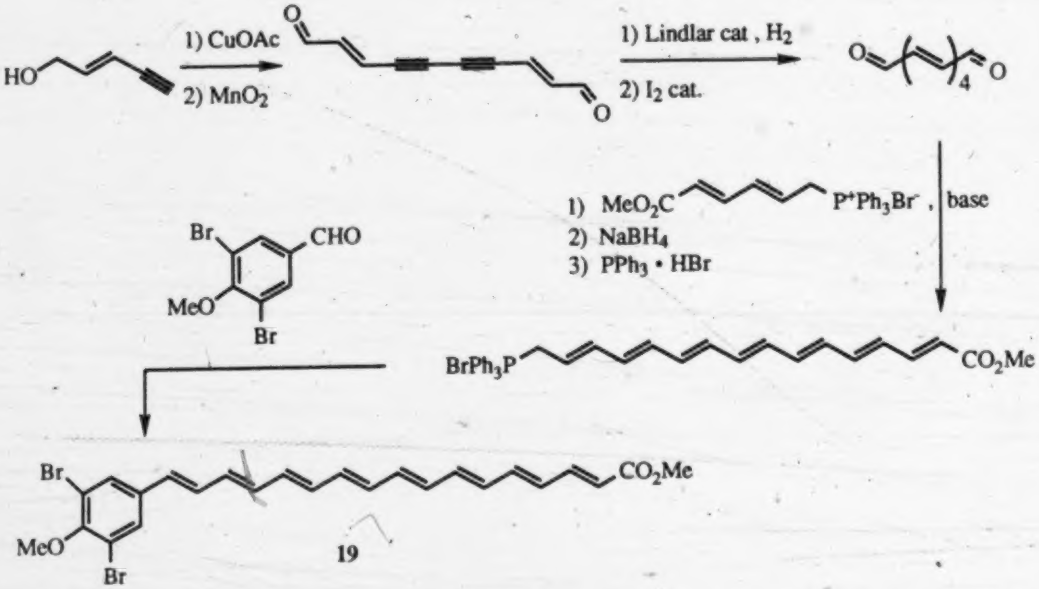
McMurry coupling is an universal tool and it has been successfully used in the synthesis of different polyenes. Again, the classical example is the synthesis of β-carotene. McMurry himself (like Wittig previously) obtained a patent[69] for this process, and there is yet another, independent report on this synthesis[70] (Eq. 3).



Eq. 3

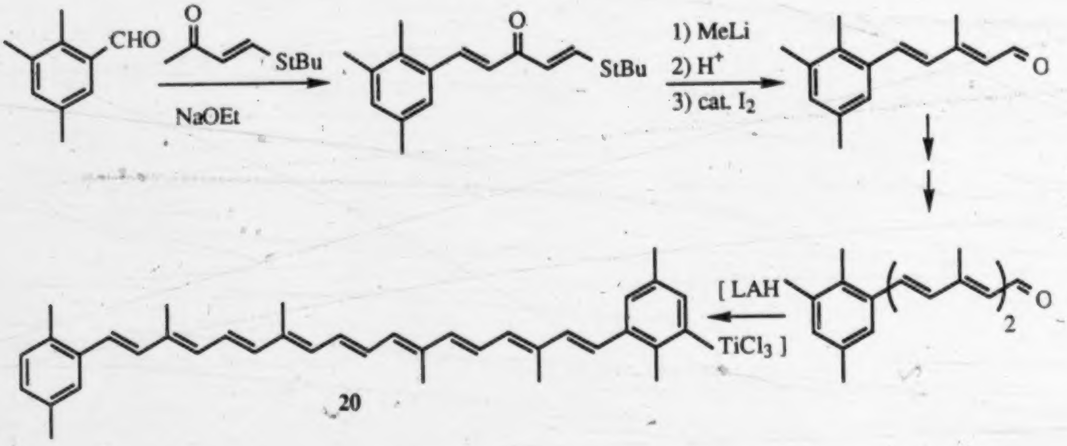
An example involving a combination of several techniques described above is the synthesis of the main pigment from the phytopathogenic bacterium, *Xanthomonas juglandis*.[71] As shown in Scheme 11, oxidative coupling of a terminal acetylene followed by hydrogenation, isomerization, and two Wittig reactions afforded material (**19**) with a system of 8 double bonds.

Scheme 11


HO
1) CuOAc
2) MnO$_2$
O
O
1) Lindlar cat, H$_2$
2) I$_2$ cat.
O
4
O
1) MeO$_2$C
P$^+$Ph$_3$Br$^-$, base
2) NaBH$_4$
3) PPh$_3$ • HBr
Br
CHO
MeO
Br
BrPh$_3$P
CO$_2$Me
Br
CO$_2$Me
MeO
Br
19

Scheme 12 shows how some classical reactions may be used

Scheme 12

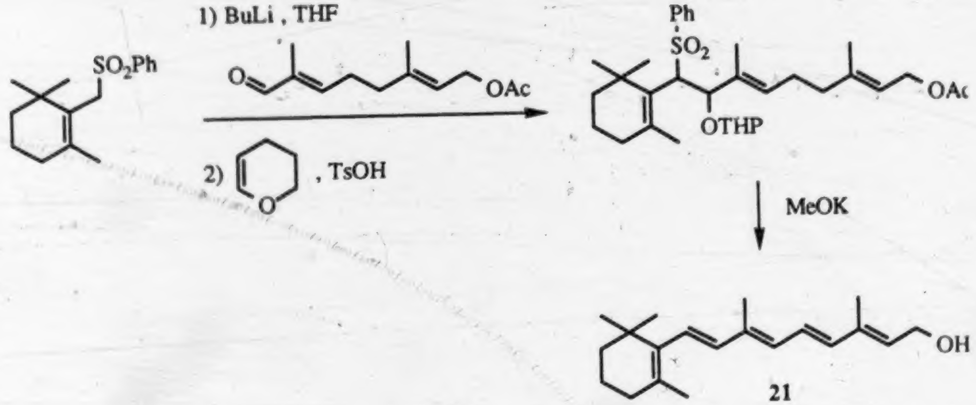

CHO
O
StBu
NaOEt
O
StBu
1) MeLi
2) H$^+$
3) cat. I$_2$
O
[LAH
TiCl$_3$]
2
O
20

in a new way. A combination of aldol reaction with the use of a novel reagent, ~~4-~~(*tert*-butylthio)-3-buten-2-one, followed by McMurry coupling was used[72] to obtain

Isorenieratene (**20**), a natural carotenoid found in certain sea sponges.

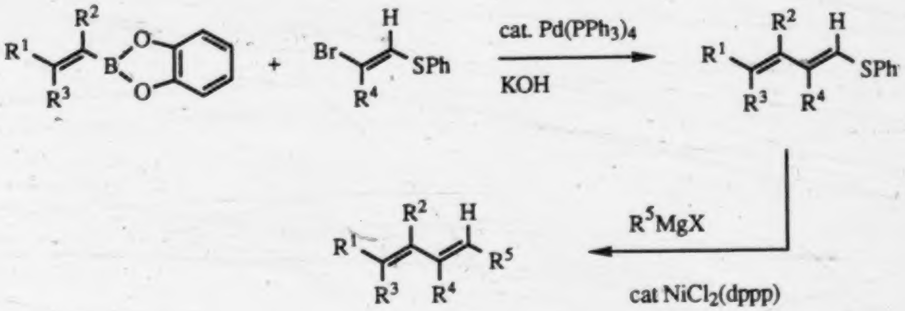
In a double elimination of α-acetoxy- or α-tetrahydropyranyloxy-β-ene sulfones, two new double bonds are formed at the same time, thus giving a conjugated triene with high trans-trans-trans selectivity. Alternatively, δ-halo-β-ene sulfones may be used. Vitamin A (**21**) was prepared in a facile way by use of this reaction[73] (Scheme 13).

Scheme 13



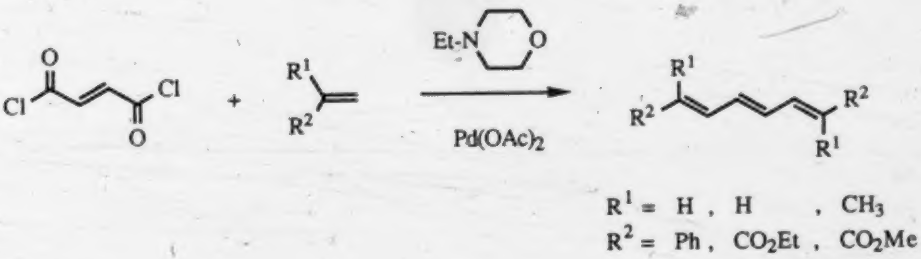
In recent years several cross-coupling reactions catalyzed by complexes of certain metals, most often palladium, and leading to formation or introduction of a double bond have been discovered. Some of these reactions were used in the synthesis of conjugated systems of double bonds. For example, cross-coupling of thiophenol derivatives of vinyl bromides with dialkoxy vinyl boranes followed by reaction with a Grignard reagent affords dienes with high stereocontrol[74] (Scheme 14).

Scheme 14


R1
R2
R3
B
O
O
+
H
Br
SPh
R4
cat. Pd(PPh3)4
KOH
R1
R2
H
SPh
R3
R4
R5MgX
cat NiCl2(dppp)
R1
R2
H
R5
R3
R4

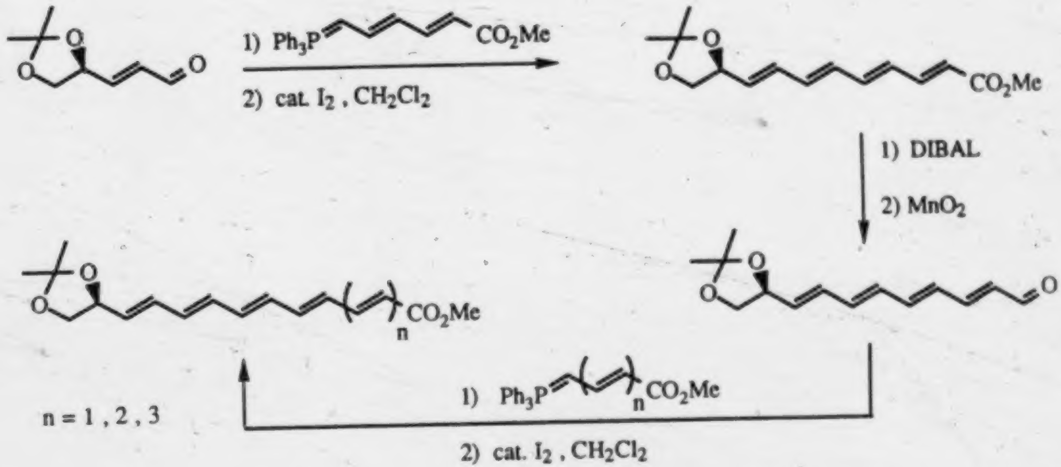
In another cross-coupling reaction, catalyzed by palladium, trienes were obtained from fumaroyl chloride (Scheme 15).[75]

Scheme 15


O
Cl
Cl
O
+
R1
R2
Et-N
O
Pd(OAc)2
R1
R2
R2
R1
R1 = H , H , CH3
R2 = Ph , CO2Et , CO2Me

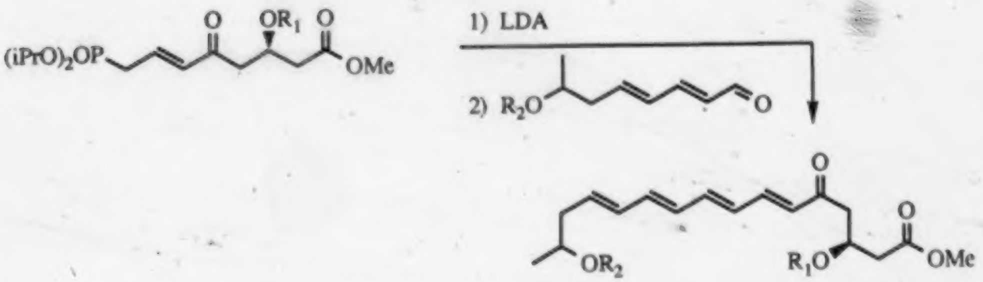
There have been several synthetic studies related to polyene antibiotics. In most cases, the Wittig reaction was used to build the polyene unit; however, other reactions were employed too. Use of unsaturated Wittig reagents followed by isomerization of the resulting material to the all-trans isomer was suggested as a good approach to build the appropriate polyene component[76] (Scheme 16).

Scheme 16


1) Ph3P CO2Me
2) cat. I2 , CH2Cl2
CO2Me
1) DIBAL
2) MnO2
O
CO2Me
n
n = 1 , 2 , 3
1) Ph3P CO2Me
2) cat. I2 , CH2Cl2

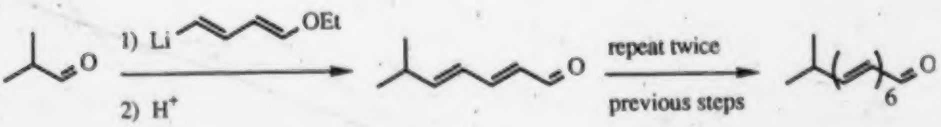
The tetraene unit of pimaricin, a polyene antibiotic which is used as a food preservative,[77] was obtained by Wittig chemistry[78] (Scheme 17).

Scheme 17


(iPrO)2OP
O
OR1
O
OMe
1) LDA
2) R2O
O
O
OR2
R1O
O
OMe

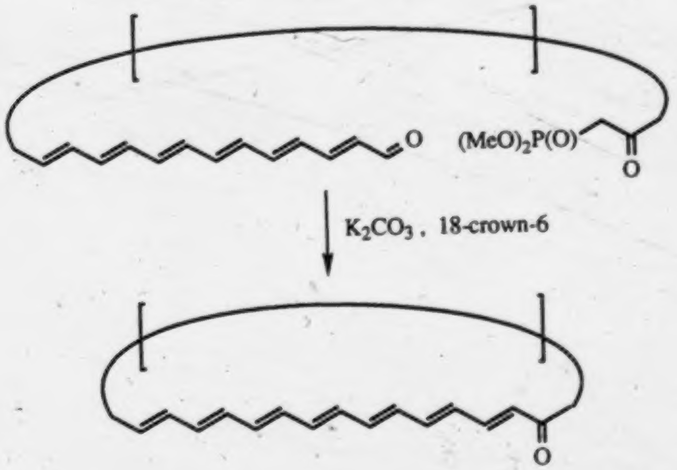
A hexaene aldehyde, which could potentially be used in the synthesis of polyene antibiotics, was obtained[79] in an iterative process with the use of Wollenberg's reagent[80] (Scheme 18).

Scheme 18


O
1) Li
OEt
2) H+
O
repeat twice
previous steps
O
6

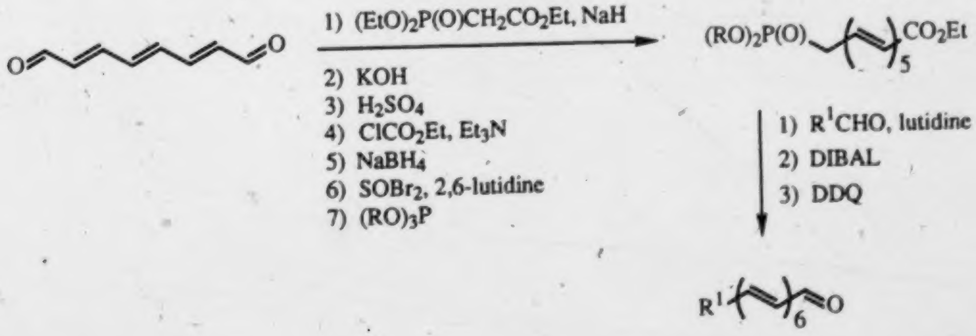
Finally, there has been one total synthesis[81] of AmB and one synthesis of the aglycone part of AmB.[82] In both cases the cyclization of the macrolide was achieved through a phosphonate olefination (Scheme 19).

Scheme 19


O
(MeO)$_2$P(O)
O
K$_2$CO$_3$, 18-crown-6
O

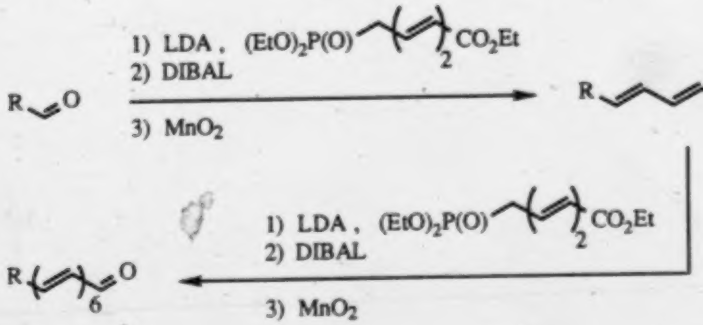
The polyene unit was assembled in each case with the help of Wittig reactions although in different ways. In one case,[83] the synthesis started with a unsaturated dialdehyde which was converted to a pentaenedioic acid ester, selectively functionalized on one terminus, and converted to the aldehyde used latter for cyclization (Scheme 20).

Scheme 20


O
O
1) (EtO)$_2$P(O)CH$_2$CO$_2$Et, NaH
2) KOH
3) H$_2$SO$_4$
4) ClCO$_2$Et, Et$_3$N
5) NaBH$_4$
6) SOBr$_2$, 2,6-lutidine
7) (RO)$_3$P
(RO)$_2$P(O)
CO$_2$Et
5
1) R^{1}CHO, lutidine
2) DIBAL
3) DDQ
R^1
6
O

In the other synthesis,[84] an unsaturated Wittig reagent was used to obtain the corresponding ester which was reduced to the alcohol, and oxidized to the aldehyde. The whole sequence was then repeated to give the requisite aldehyde (Scheme 21).

Scheme 21



As all the above examples show, there are a number of reactions available for the synthesis of polyenes, and a broad selection of conditions may be used. It seems, however, that the reaction that has been utilized most is the Wittig olefination. The modifications developed over the years allow for greater control over the reactivity of the reagent used, enable better stereocontrol, and above all, allow for the formation of double bond under very mild conditions. This particular feature is of great importance in complex synthesis as witnessed by the last examples.

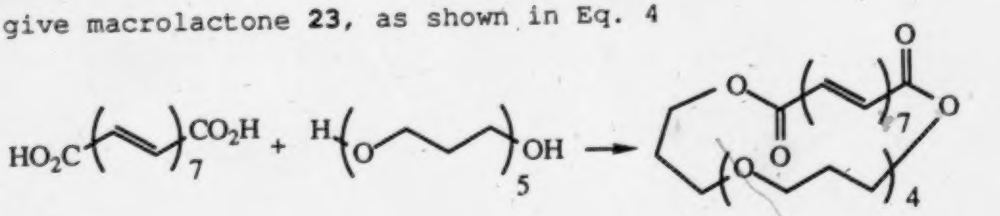
II. RESULTS AND DISCUSSION

II.1 Introduction.

The aim of the present work was to use the methods of synthesis to explore the essential structural requirements for biological activity of the polyene macrocycle, Amphotericin B. This is the most important member of its compound class and has the ability to form channels through membranes. It is not known what structural features of the molecule endow it with this property, although there has been much speculation, as described in the previous chapter. If it is possible to build a molecule possessing some of the structural features of the natural product while retaining its channel-forming ability, then such an exercise would serve to identify the structural requirements for channel formation. Since Amphotericin B is an important antifungal agent, an understanding of its mode of action could have important practical consequences in medicine.

The gross features of the natural product are that it possesses a rigid hydrocarbon spine, the polyene unit, which is of appropriate length to span one half of a membrane bilayer. The ends of this rigid unit are joined by a hydrophilic polyhydroxy chain. Attached to the resulting structure is an amino sugar and a carboxyl group. No extensive structure—activity work had been reported in this area when we began, and so we felt that more detailed analysis of the structural features of Amphotericin B was unwarranted at this stage. In order to evaluate which of the gross features are necessary, our plan was to construct a molecule of suitable dimensions, and carrying, as does the natural product, a hydrophobic chain spanned by a hydrophilic chain. Because of the structural simplicity of the polyene carbon backbone of the natural product, it seemed appropriate to imitate that unit completely and incorporate it into our model compounds. It would, of course, have a suitable length, and so the primary choice was in the nature of the hydrophilic unit designed to span the ends of this polyene system. The constitutional requirements for this unit are first that it has water solubility and that it be the right length. However, the corresponding segment of the natural product has a more subtle characteristic. If the molecules are going to form channels, then two criteria should be satisfied: once the channel is formed the constitution of the cluster should be such as to stabilize it, but in addition, a mechanism should be available for individual molecules to undergo self-assembly. The polyhydroxy substituents of the natural product satisfy these criteria; once the cluster is formed intermolecular hydrogen bonding could stabilize it and the same phenomenon of intermolecular hydrogen bonding could also serve as a mechanism by which the individual molecules could become organized. At the first level of approximation, at which we began this work, we decided not to include substituents that would facilitate self-assembly but to incorporate only substituents that might serve, by means of hydrogen bonding with bridging water molecules, to stabilize the unit once it is assembled. With this admittedly gross simplification, the possibility arose of using a polyether to span the ends of the polyene system, and examination of space filling models of the CPK type suggested that 1,3-polyethers are appropriate, because in the extended form all oxygen atoms are oriented in a direction to make them easily accessible for the required interaction with water (Fig. 3). Later on, we were to examine corresponding polythio compounds. The reasoning behind that choice is that the polythio structure would offer an opportunity for oxidation to a polysulfoxide, and polysulfoxides do incorporate a feature to facilitate self-assembly. In addition, a polysulfide might be oxidized *in vivo* to the polysulfoxide level so that our model would serve as a prodrug.

There are in principle, a number of ways in which a polyether and a heptaene could be built into a macrocycle. Our initial plan was that the ring would be closed by macrolactonization, and on this basis, the component units that are required are diacid **11** and diol **22**. These should give macrolactone **23**, as shown in Eq. 4



11 **22** **23**

Eq. 4

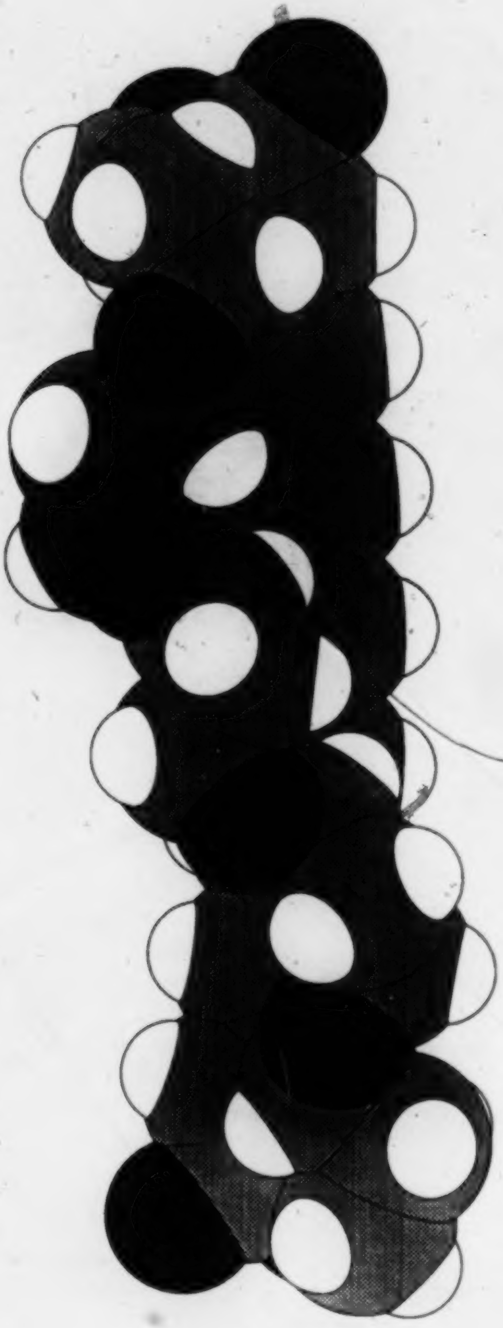
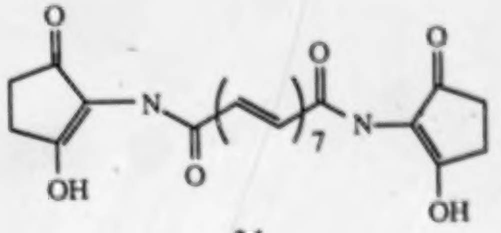


Fig. 3. CPK model of lactone **23**.

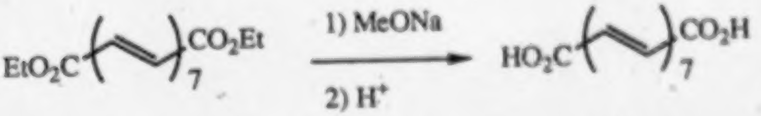
II.2. Synthesis of heptaenedioic acid 11.

Diacid **11**, is a known substance,^{53} named Descrocetin (after its tetramethyl analog). There is at least one natural product which is a derivative of **11**, Limocrocin



24

24, a yellow pigment which was isolated[85] from the culture liquid of *Streptomyces limosus*. Apparently, direct conversion of **24** to **11** is difficult because originally, limocrocin was assigned its structure only after hydrogenation and cleavage of the saturated derivative. Unfortunately, the only reference to a synthesis of diacid **11** is in the old literature.^{53} The compound was obtained by hydrolysis of the corresponding diester **16** (Eq. 5) but the reported method

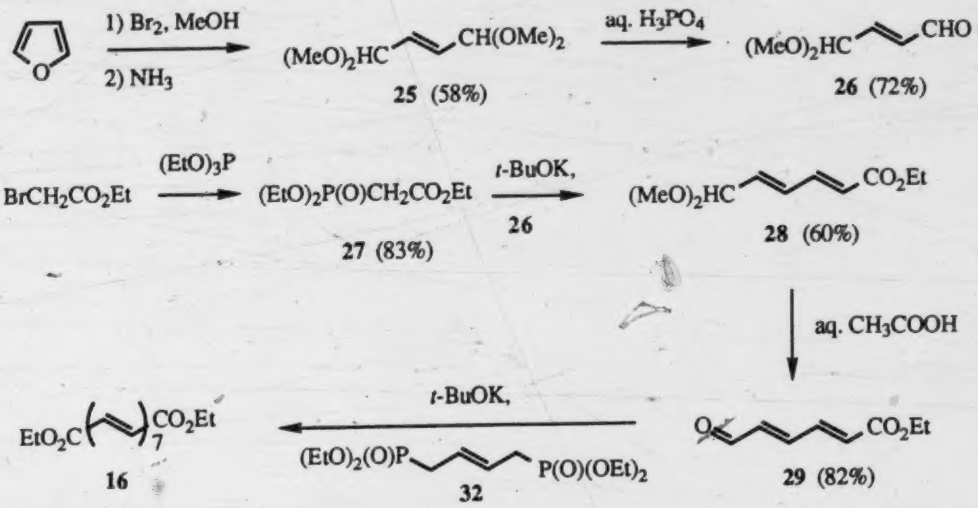


Eq. 5

16 **11**

gives diester **16** in very low yield (after a series of aldol condensations).

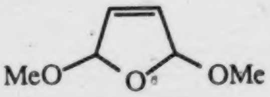
Scheme 22


1) Br_2, MeOH
2) NH_3
$(MeO)_2HC$
$CH(OMe)_2$
25 (58%)
aq. H_3PO_4
CHO
26 (72%)
$BrCH_2CO_2Et$
$(EtO)_3P$
$(EtO)_2P(O)CH_2CO_2Et$
27 (83%)
t-BuOK,
26
CO_2Et
28 (60%)
aq. CH_3COOH
O
29 (82%)
t-BuOK,
$(EtO)_2(O)P$
$P(O)(OEt)_2$
32
EtO_2C
7
16

In the more recent literature there is a reference to diester **16** which describes the synthesis of **16** based on Wittig reactions.[66] However, the yield is again below 10%. A significantly better route was clearly needed, and to this end, our original plan was the one shown in Scheme 22. In the early stages of the project the readily available aldehyde **29** caught our attention as a potentially useful starting material.[a] Aldehyde **29** could clearly serve as a precursor to diester **16** (and hence to diacid **11**) by double Wittig condensation with the known diphosphonate **32**. The aldehyde **29** was obtained by the literature route[86,87] summarized in Scheme 22. Treatment of furan with bromine in methanol and then with ammonia gave diacetal **25** in good yield. Because there are conflicting differences between the literature procedures,[88,89] we repeated this experiment several times and found that the reaction is quite sensitive to the experimental conditions. Depending on the temperature, various ratios of diacetal **25** and dimethoxydihydrofuran **30** are obtained.

---

[a] Coupling two molecules of structure **8** by the McMurry reaction would provide a pentaenedioate that could be used for analogous structure—activity studies related to Filipin, another polyene macrolide that is shorter than amphotericin B. There are some indications[9] that the lower activity of Filipin, compared to amphotericin B, is attributable to its shorter length.



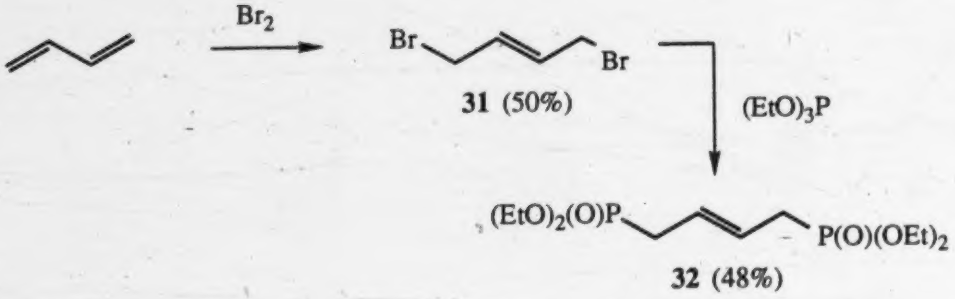
30

We determined that the optimum conditions were those that had already been reported[90] and this was subsequently found also by others.[91]

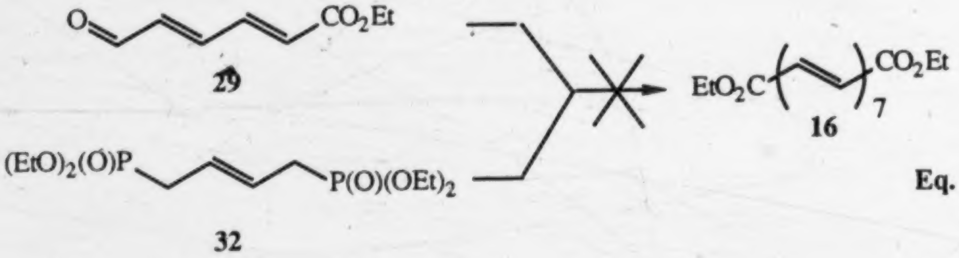
The acetal **25** was selectively cleaved to monoaldehyde **26**. Triethyl phosphonoacetate **27** was made by Arbusov reaction between triethyl phosphite and ethyl bromoacetate and was used in the Wittig reaction with monoaldehyde **26**. This process gave acetal **28**, which was hydrolyzed to aldehyde **29** (Scheme 22).

The other Wittig component (**32**) required for the synthesis of **16** was obtained as shown in Scheme 23.

Scheme 23


Br$_2$
Br
Br
31 (50%)
(EtO)$_3$P
(EtO)$_2$(O)P
P(O)(OEt)$_2$
32 (48%)

Addition of bromine to butadiene gave 1,4-dibromobut-2-ene **31**, and on treatment with ethyl phosphite, this afforded **32**. Unfortunately, our attempts to couple aldehyde **29** with diphosphonate **32** (Eq. 6) were unsuccessful.

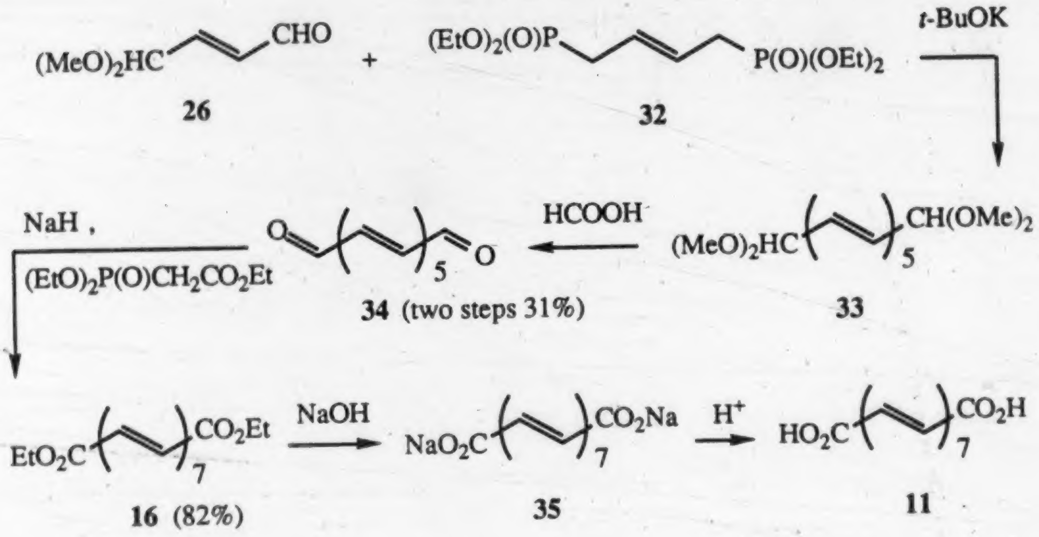

O
CO$_2$Et
29
(EtO)$_2$(O)P
P(O)(OEt)$_2$
32
EtO$_2$C
CO$_2$Et
7
16

Eq. 6

We tried n-butyllithium in THF, potassium *tert*-butoxide in THF and potassium *tert*-butoxide in toluene, but under all of these conditions we obtained only inseparable mixtures. We did not try sodium hydride in the presence of 18-crown-6.

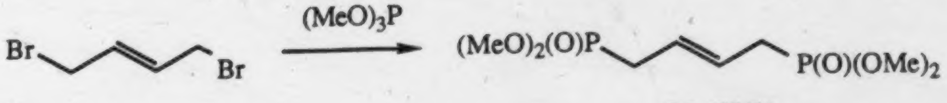
We therefore examined an alternative way (Scheme 24) of assembling the diester **16**, making use of reagents that had already been prepared.

Scheme 24


(MeO)2HC
CHO
26
+
(EtO)2(O)P
P(O)(OEt)2
32
t-BuOK
(MeO)2HC
CH(OMe)2
5
33
HCOOH
O
O
5
34 (two steps 31%)
NaH ,
(EtO)2P(O)CH2CO2Et
EtO2C
CO2Et
7
16 (82%)
NaOH
NaO2C
CO2Na
7
35
H+
HO2C
CO2H
7
11

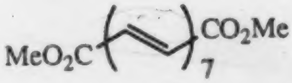
Aldehyde **26** was coupled with phosphonate **32** to diacetal **33**, and hydrolysis then afforded dialdehyde **34**. The diacetal **33** is quite unstable and easily gives polymeric materials. Consequently, the yield in the coupling (**26** + **32**->**33**) was rather low. We decided to cleave the diacetal **33** without attempting to purify it first. In sharp contrast to **33**, the parent dialdehyde **34** is stable and may be stored at low temperature (*ca* 0°C) for several months without noticeable deterioration.

Later on, we found that **36**, the methyl analog of **32**, is a solid at room temperature and is easier to purify. It was prepared (Eq. 7) in a similar way to **32**, using dibromide **31** and trimethyl phosphite.



31 **36** (53%) **Eq. 7**

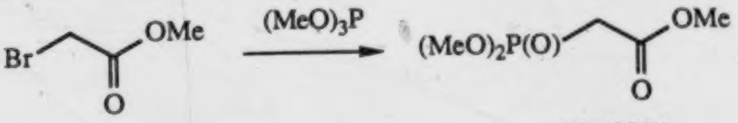
Finally, the sequence of Scheme 24 was completed by coupling dialdehyde **34** with triethyl phosphonoacetate **27** to afford the diester **16**. The literature procedure[53] for hydrolysis of **16** was then applied (sodium methoxide in dioxane–methanol mixture). However, the very low solubility of the starting diester in this system, as well as the fact that on one occasion instead of the expected sodium salt of the diacid, its dimethyl ester **37** was obtained, prompted



37

us to look for other systems. Use of anhydrous potassium *tert*-butoxide[92] in dioxane failed and the starting material was recovered. The same reagent in hot toluene (which dissolves the diethyl ester quite well) did not succeed either, and decomposition was observed. Boron tribromide[93] in dichloromethane gave recovered starting material as well as some black tarry products. Finally, ethanolic potassium hydroxide in hot toluene followed by aqueous hydrochloric acid was found to give acceptable results, although the product was slightly contaminated with mono ester, as judged by the presence of carboethoxy signals in the NMR spectrum of the material.

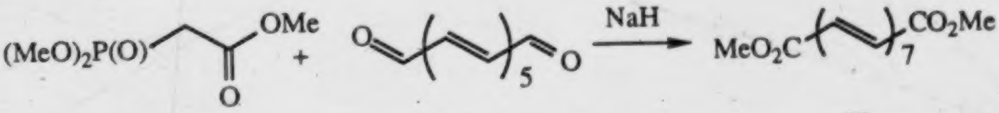
Difficulties in hydrolyzing the diethyl ester prompted us to synthesize the dimethyl analogue (**37**) in the expectation that this would be easier to cleave. Accordingly, trimethyl phosphonoacetate **38**, obtained from



38 (90%)

Eq. 8

methyl bromoacetate and methyl phosphite (eq 8), was coupled with aldehyde **34** (eq. 9). However, the poor solubility of the dimethyl ester **37** precluded its further use.



38 **34** **37** **Eq. 9**

It is known[94,95] that *tert*-butyl esters of carboxylic acids can be cleaved under relatively mild acidic conditions, so we decided to explore this possibility. The synthesis of acid **11** by this route is shown on Scheme 25.

Chloracetic acid chloride was converted into *tert*-butyl chloroacetate **39** with *tert*-butyl alcohol and triethylamine. An Arbusov reaction of **39** with methyl phosphite then gave *tert*-butyl dimethyl phosphonoacetate **40**.[a]

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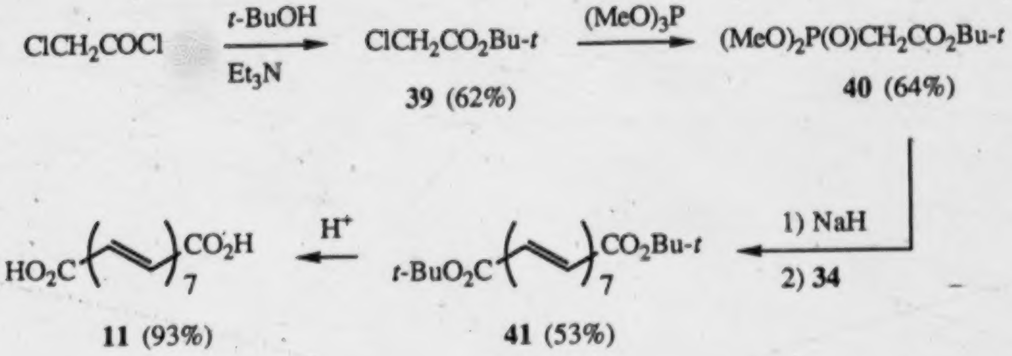
[a] We noticed that under the reaction conditions an exchange of *O*-alkyl groups between the carboxylic ester and the phosphite occurs quite

Scheme 25


ClCH2COCl
t-BuOH
Et3N
ClCH2CO2Bu-t
39 (62%)
(MeO)3P
(MeO)2P(O)CH2CO2Bu-t
40 (64%)
1) NaH
2) 34
t-BuO2C
CO2Bu-t
7
41 (53%)
H+
HO2C
CO2H
7
11 (93%)

Under the conditions used previously for the synthesis of diethyl and dimethyl esters, the *tert*-butyl phosphonate **40** gave the di-*tert*-butyl diester **41**, and this compound, on treatment with *p*-toluenesulphonic acid in boiling benzene afforded diacid **11** in 93% yield. The compound is an orange, crystalline material.

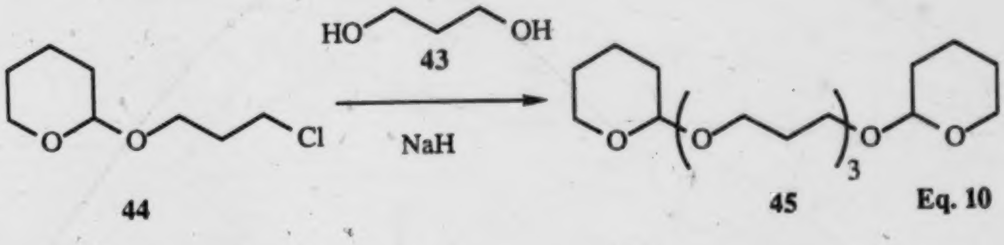
II.3. Synthesis of diol 22.

Surprisingly, there is no reference in the chemical literature to diol **22**, but mixtures of oligomeric

---

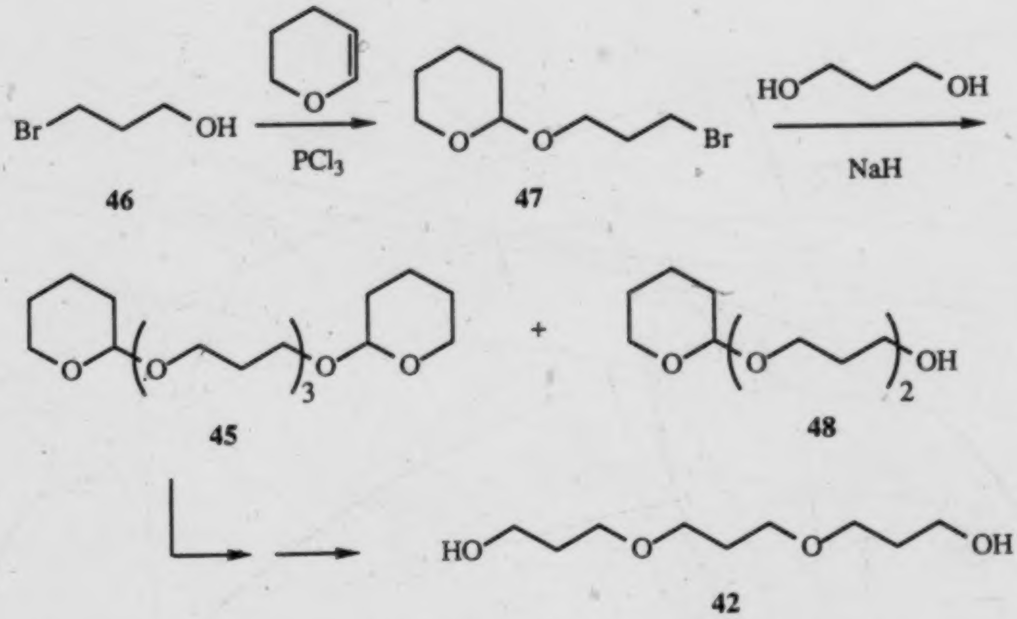
readily. As a result, apart from the expected t-butyl derivative, 5–10% trimethyl phosphonoacetate **38** was obtained as well in this process. Similar observations were made when when we carried out a reaction between methyl bromoacetate and triethyl phosphite, some 10% of by-product, most likely (^{1}H NMR) triethyl phosphonoacetate was detected.

trimethylene polyether diols are known and have some industrial applications.[96] A lower homologue of **22**, the trimeric diol **42** (see page 51), has been reported.[97] It was obtained by alkylation of trimethylene diol **43** with the protected chloropropanol **44** (Eq. 10) followed by removal of the protecting groups. We hoped that by repeating the alkylation-deprotection steps, the sequence could be extended to produce the pentameric diol **22**. However, such a plan has serious disadvantages. The overall yield of the transformations involved (**46**->**45**->**22**, Scheme 26)[97] is quite low (23%) so a very low yield of the pentameric diol **22** would be expected. Nevertheless, we did repeat the literature procedure on a smaller scale, using as starting material bromopropanol **46** (Scheme 26), in the hope that the higher activity of bromide versus chloride would result in a better yield (see Eq. 10).



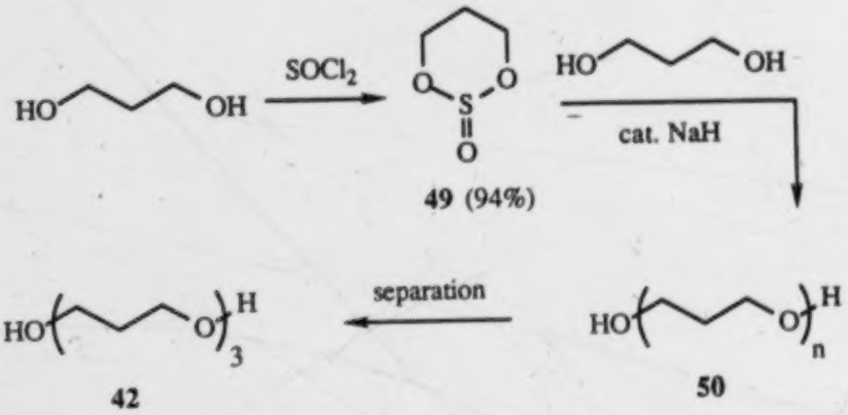
Eq. 10

Scheme 26



We found that the low overall yield is a result of difficulties at the alkylation step (**47**->**45**), because a mixture of mono- (**48**) and di-alkylated (**45**) products was obtained. We suspected that the polyoxygenated intermediate **48** resembles a crown ether and may in a similar fashion chelate sodium ions. A chelated RO^- species would, of course, also have a decreased nucleophilicity. The reaction was run in DME (dimethoxyethane) with sodium hydride, and the terminal alkoxide unit is sterically inaccessible as it is likely to be affiliated with intramolecularly chelated sodium ions. To try to circumvent this problem metal ions should be avoided, so an experiment was conducted by a phase transfer method (ether, tetrabutylammonium bromide, 50% aqueous soddium hydroxide). However, under these conditions the alkylating agent underwent hydrolysis, and an even more complex mixture was obtained.

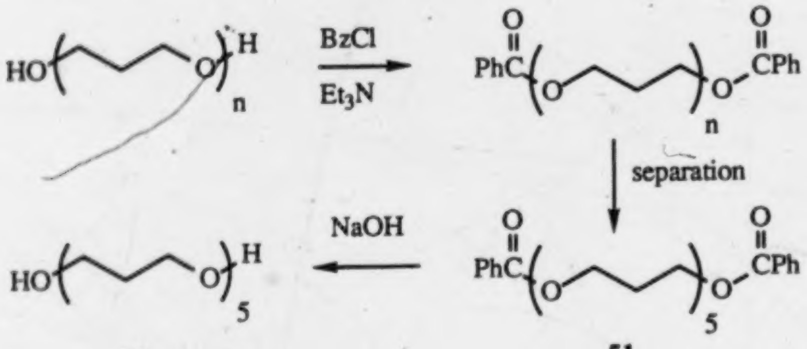
Scheme 27



In view of these observations we decided to make **42** by the route shown in Scheme 27.

Trimethylene sulfite **49** was prepared according to a literature procedure[98] and was subjected to the reported oligomerization with trimethylene diol.[96] Distillation of the reaction mixture under reduced pressure did not totally separate the lower components although trimeric diol **42**, needed for the alkylation, could be isolated in a state of about 90% purity (V.P.C.). We realized at this point that the alkylation could be avoided by simply isolating the pentameric diol **22** from the polymerization mixture (Scheme 28).

Scheme 28



22 **51**

The oligomerization was repeated several times using triphenyl phosphite (tributyl phosphite was used in the literature procedure), phosphoric acid, or sodium hydride as the catalyst. We did not find any significant differences in the results and we used sodium hydride in subsequent runs. The initial ratio of trimethylene sulfite **49** to trimethylene diol was 2:1. Increased ratios and prolonged heating times resulted in large amounts of higher oligomers. Dimeric and trimeric products were removed by distillation under reduced pressure (*ca* 1 mm) and distillation under very low pressure (below 0.001 mm) afforded a mixture of tetra-, penta-, and hexameric diols. Attempts to separate this mixture by flash chromatography or preparative HPLC were unsuccessful. Fortunately, after acetylation we were able to separate the mixture into its individual components. For a practical reason, benzoylation was chosen, as acetates were poorly visibly on TLC while benzoates were easy to detect under UV light. A mixture of benzoates was prepared and the pentameric dibenzoate **51** was separated. Diol **22** was then obtained from **51** by hydrolysis or reduction with lithium aluminum hydride.

II.4. Attempts to esterify diacid 11 and to obtain the macrolactone with diol 22.

With the pentameric diol in hand, the next step in the assembly of an Amphotericin B model involved esterification of diacid **11** with the diol **22**.

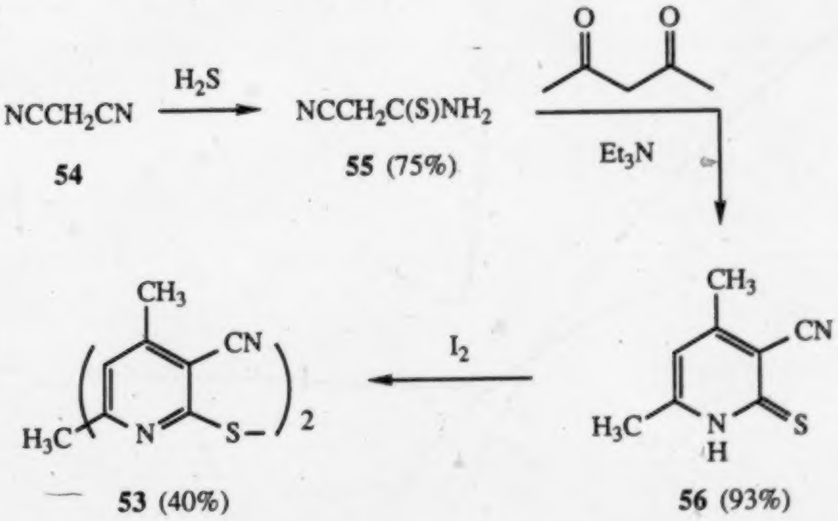
We decided to first optimize the conditions for esterification of diacid **11** using isopropanol as the alcohol component. This is a secondary alcohol and would not, therefore, underrepresent the steric situation present in our diol. Moreover, conditions that worked for a secondary alcohol would be expected to be suitable also for a secondary diol. This point is relevant to our further plans as we hoped to functionalize the diol component at the hydroxyl-bearing carbon.

Several attempts to esterify diacid **11** were unsuccessful. Reaction with isopropyl alcohol in benzene under reflux and removal of water with molecular sieves failed, and the use of isopropyl alcohol in the presence of

DCC, DMAP, and a catalytic amount of *p*-toluenesulphonic acid[99] also did not give the expected diester. We then tried to use the complex of triethyl phosphite with iodine in the presence of triethylamine[100] and isopropyl alcohol, but this reagent also did not effect esterification.

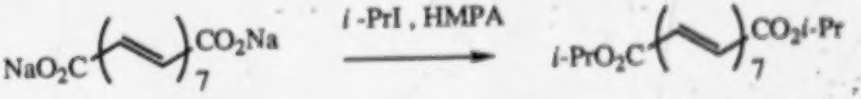
Attempts to achieve esterification by activating the diacid were likewise unsuccessful. 2-Chloromethylpyridinium iodide **52** did not bring about esterification, so an even more powerful activating reagent (**53**) was prepared by the series of reactions (**54->55->56->53**) shown in Scheme 29.

Scheme 29



Unfortunately, application of this reagent did not afford the desired diisopropyl ester, and the diacid **11** remained unchanged.

We next turned our attention to esterification techniques based on use of alkyl halides and we tried to esterify diacid **11** with isopropyl iodide in the presence of DBU (diazabicycloundecane). However, no reaction occurred under these conditions. Use of the disodium salt of diacid **11** and isopropyl iodide in the presence of tetrabutylammonium hydrogen sulfate was equally unsuccessful. However, we were finally able to obtain the diisopropyl ester **57**, although in very low yield, by reaction of the disodium salt **35** in HMPA (hexamethylphosphoramide) with a large excess of isopropyl iodide (Eq. 11).

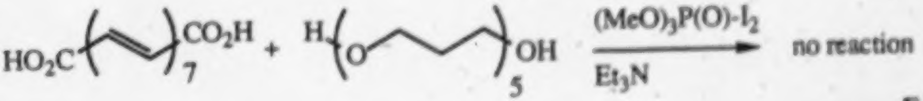


35

57 (10%)

Eq. 11

Although the model experiment with isopropanol did not work, we took a sample of diol **22** and attempted lactonisation with diacid **11** in the presence of trimethyl phosphite–iodine complex and triethylamine (Eq. 12).



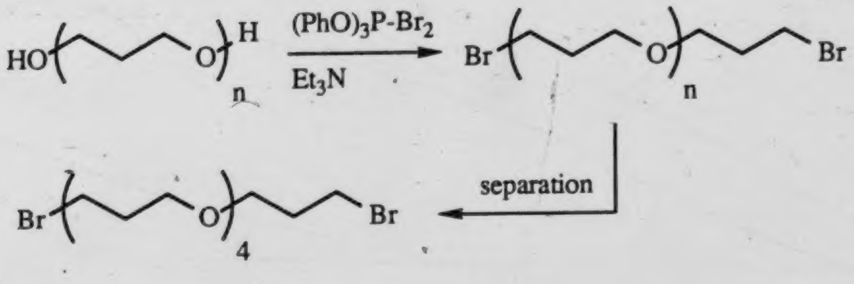
11

22

Eq. 12

None of the expected product could be detected. As the only conditions under which we observed formation of a diisopropyl ester involved use of isopropyl iodide, we had to convert diol 22 into the corresponding dibromide 58, which would then be used for macrolactonization.

Scheme 30

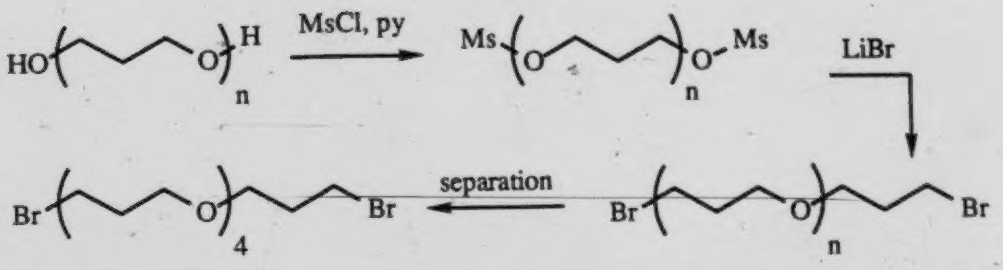


58

A mixture of tri- to hexameric diols was converted into a corresponding mixture of dibromides by the use of triphenyl phosphite–bromine complex in the presence of triethylamine (Scheme 30).

The action of triphenylphosphine and carbon tetrabromide was also suitable, and the individual dibromides were then separated by flash chromatography. However, because it was difficult to use stoichiometric amounts of reagents (as we could only approximate the composition of the diol mixture), a number of byproducts were formed and the overall yield was rather low. To remedy this problem, the mixture of diols was first mesylated and the resulting mesylate mixture was converted into a mixture of bromides by reaction with lithium bromide in refluxing isopropyl alcohol (Scheme 31).

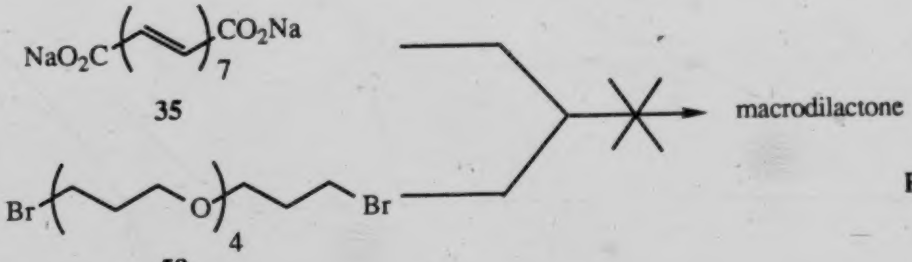
Scheme 31


MsCl, py
LiBr
separation
HO
H
n
Ms
Ms
O
Br
4

58

Flash chromatography afforded the dibromide **58**, which was subsequently used in attempts to obtain the macrolactone.

All attempts to effect macrolactonization were carried out in HMPA but none was successful. Diacid **11** and dibromide **58** did not cyclize in the presence of tetrabutylammonium hydroxide[101] or in the presence of DBU. Reaction in the presence of cesium carbonate was unsuccessful, and so was the reaction between disodium salt **35** and dibromide **58** (Eq. 13).

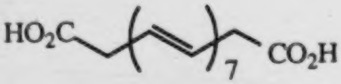

NaO$_2$C
CO$_2$Na
7
35
Br
O
4
Br
macrodilactone

58

Eq. 13

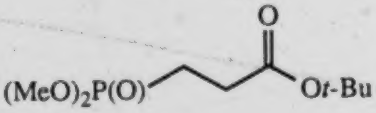
II.5. Attempts to generate the macrolactone in alternate process.

It was not clear why diacid **11** is so difficult to esterify and we suspected that the conjugated nature of the carboxyl groups is responsible for the problem. In order to test this hypothesis we decided to prepare a similar diacid (**59**) containing a heptaene unit but also having the carboxylic groups isolated.



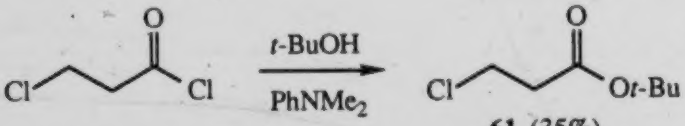
59

We hoped that an appropriate diacid would be accessible by repeating the previous procedure but using a different Wittig reagent, specifically compound **60**.



60

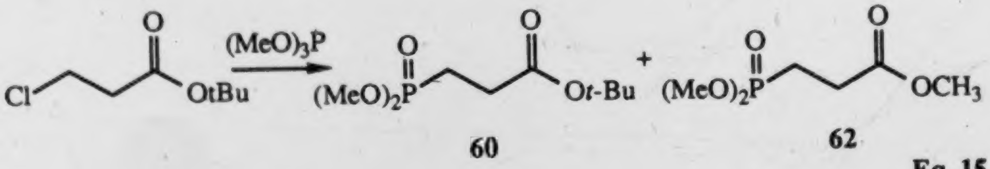
We tried to prepare this reagent in the following way.



61 (35%)

Eq. 14

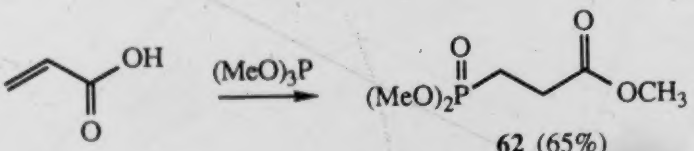
2-Chloropropionyl chloride was converted into *tert*-butyl 2-chloropropionate **61** (Eq 14) and this was refluxed with methyl phosphite in the expectation of obtaining the *tert*-butyl ester **60**. However, an exchange of *O*-alkyl groups, which we had observed earlier, occured once again, and in this case was the predominant process, with the main product of the reaction being the methyl ester **62** (Eq. 15)



60 **62**

Eq. 15

As at this point we were interested to find out if **62** and similar types of reagent would couple with pentaene dialdehyde **34**. We prepared pure **62** efficiently from methyl phosphite and acrylic acid[102] (Eq. 16).



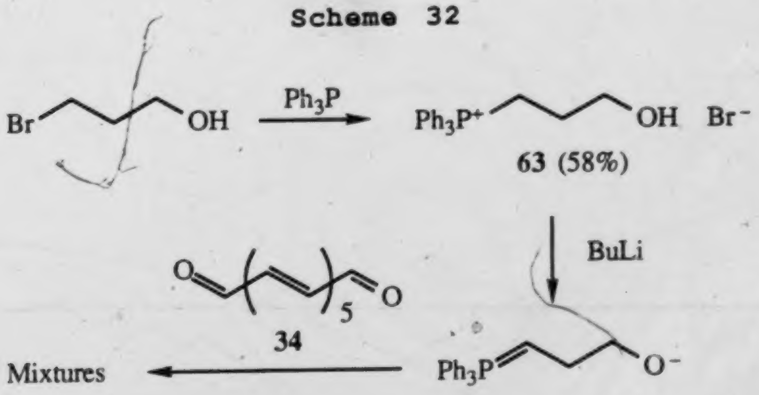
Eq. 16

62 (65%)

Unfortunately, we were unable to carry out the Wittig reaction between **62** and **34** because we always obtained mixtures in which we could not detect the expected condensation product.

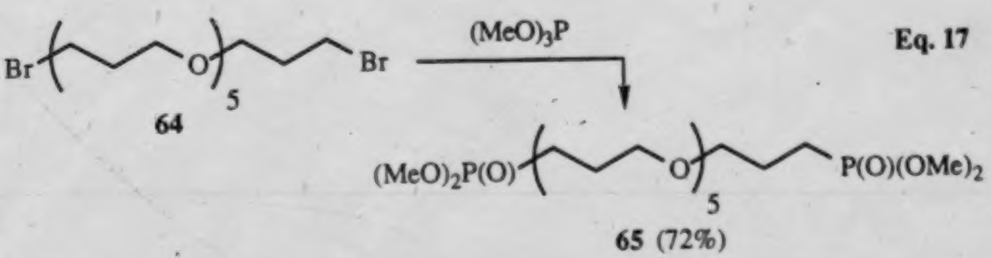
An alternative approach to the type of macrocyclic lactones we wanted could involve placing the hydroxyl groups on the heptaene and the carboxyls on the polyether. To implement this idea, a Wittig reagent **63** possessing a hydroxyl group[103] was prepared from triphenylphosphine and 3-bromopropan-1-ol (Scheme 32) but again, attempts to couple this reagent with dialdehyde **34** with the use of butyllithium or sodium hydride as a base, led to intractable mixtures.

Scheme 32



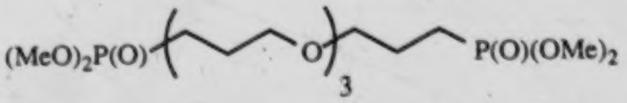
II.6. Use of the Wittig reaction and cyclization of modified model compounds.

The unanticipated obstacles to macrodilactonization caused us to make a further change in our general approach. We decided to explore the possibility of employing the Wittig reaction itself as a means of closing the macrocycle. This idea represents only a slight departure from the original plan in the sense that the structural features of the original model compound would be retained. Examination of molecular models indicated that with the polyether unit as the hydrophilic component of the macrocycle, the hexameric dibromide **64** should be used to obtain a Wittig reagent **65** of appropriate length (see Eq. 17). Fortunately, we already had the dibromide in hand as it had been separated along with the pentameric dibromide from the mixture of dibromides described above.

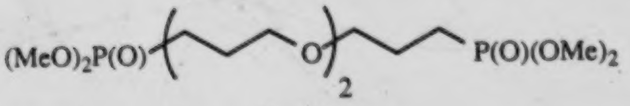


Eq. 17

Dibromide **64** afforded diphosphonate **65** (Eq. 17) on reaction with trimethyl phosphite. Attempts to cyclize our pentaene dialdehyde **34** with diphosphonate **65** were, however, unsuccessful. Reaction with potassium *tert*-butoxide gave only very small amounts of unidentified products and unchanged aldehyde **34**, while use of sodium hydride led to formation of a complex mixture of very polar materials. We tried to condense **65** with benzaldehyde, using butyllithium, but without success, so we conclude that **65** is an unsuitable phosphonate for Wittig reactions with conjugated aldehydes. Two shorter analogs of **65** were also prepared—the tetrameric diphosphonate **66** and the trimeric diphosphonate **67**, but attempts to condense even these materials with benzaldehyde with use of butyllithium or sodium hydride were not successful (mixtures of very polar materials were obtained).



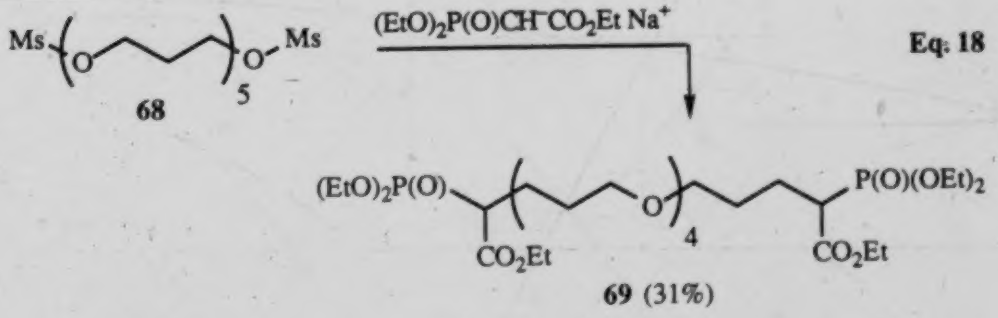
66



67

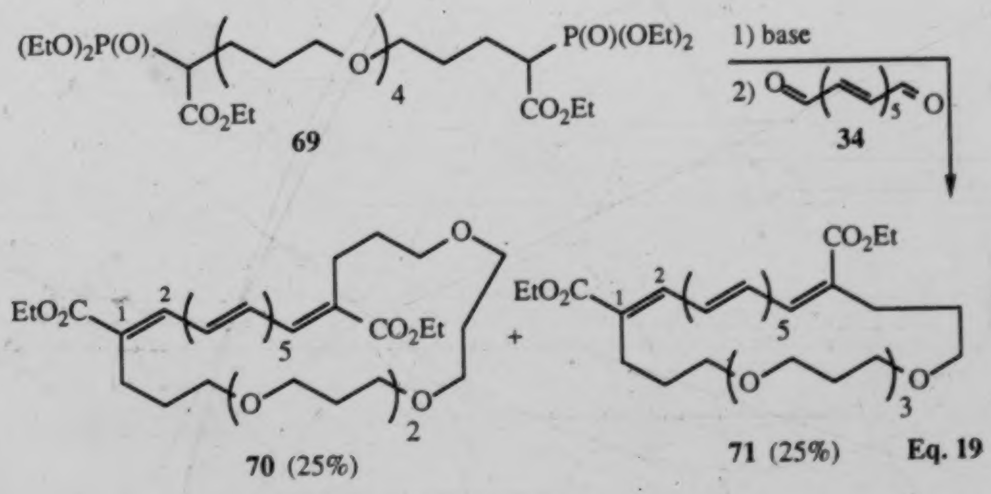
At this point we came to the conclusion that the fact that pentaene dialdehyde **34** condenses so easily with certain phosphonoacetates (as observed in preparing the heptaene diacid) could most likely be attributed to a delicate balance between the basicity of the doubly stabilized anion in the phosphonate and the reactivity of the conjugated aldehyde. On this basis a modification of phosphonate **65** was necessary so as to incorporate carboalkoxy groups, appropriately placed to lower the basicity of the bis(phosphonate) anion. The incorporation of ester groups would also offer potential sites for additional transformations after ring closure, such as attachment of amino acids or carbohydrates.

One way to obtain an ester-substituted phosphonate of appropriate length would be by alkylation of pentameric dibromide **58** with the sodium salt of triethyl phosphonoacetate. We tried this reaction but it was very slow, starting materials were recovered, and only small quantities of other materials were formed. We decided that the more reactive mesylate **68** was needed. It was isolated from the mixture of mesylates used previously to obtain the corresponding bromides, and this route did afford the requisite doubly-stabilized bis-Wittig reagent **69**, although in low, but acceptable, yield (Eq. 18).


Ms
O
O
Ms
5
68
$(EtO)_2P(O)CH^-CO_2Et\ Na^+$
$(EtO)_2P(O)$
O
4
$P(O)(OEt)_2$
CO_2Et
CO_2Et
69 (31%)

Eq. 18

The diphosphonate **69** did indeed react with dialdehyde **34** under conditions of high dilution (*ca* 2 mmol/L) to give two isomers of cyclized material, **70** and **71** (Eq. 19).


$(EtO)_2P(O)$
O
4
$P(O)(OEt)_2$
CO_2Et
CO_2Et
69
1) base
2) O O
5
34
EtO_2C
1
2
5
CO_2Et
O
O
O
2
70 (25%)
+
CO_2Et
EtO_2C
1
2
5
O
O
3
71 (25%)

Eq. 19

The reaction was tried with several bases such as butyllithium and sodium hydride, but we found that the most efficient reaction occured with lithium bis(trimethylsilyl)amide. The yield of the reaction, although not unprecedented in cyclizations to rings of 30 or more atoms, was very high, and we were able to approach 50% total yield for both isomers. The two isomers differed in their properties. The less polar (**70**) was found to be quite stable; on storage in the dark for several months only partial decomposition was observed for solid samples and solutions were even more stable. However, exposure of the substance to light caused appreciable decomposition after several days. The more polar isomer (**71**) was much less stable, and in fact, we were not able to obtain this material in its pure form. It partially decomposed and/or isomerized to **70** during separation on silica. We found that isomerization of **71** to **70** can be accomplished by gentle heating of a dichloromethane solution of **71** with catalytic amounts of iodine, a procedure used for isomerization of conjugated double bonds[76] to an all-trans system. The isomerization was not complete, however, and a portion of the material underwent polymerization. Attempts to use UV light to accomplish this isomerization led invariably to total disappearance of starting material in a few minutes.

In general all-trans systems are more stable than isomers containing cis double bonds and this fact suggests that **70** has the all-trans geometry. The high field carbon

NMR spectrum of **70** showed that it is a symmetrical molecule, while the high field proton NMR spectrum revealed that the C(3)–C(4) double bond is most likely trans (^3J = 14.5 Hz). Comparison of the high field proton NMR spectra of **70** and **71** showed that the only difference between the two isomers is the geometry of one of the newly formed (terminal) double bonds of the heptaene system. In compound **70**, the signal corresponding to the allyl protons is a binomial triplet with integration corresponding to 4 hydrogens, while in **71**, the equivalent signals are composed of two triplets, each corresponding to 2 hydrogen atoms. These features indicate that in **71** one of the terminal bonds is trans and the other is cis. As allylic coupling is usually very small and not indicative of double bond geometry the only evidence to assign a trans disposition to the terminal double bond in **70** (except for the above-mentioned observation that it was the more stable isomer)[a] was the chemical shifts of the α and β (Fig. 4) protons of the polyene system.

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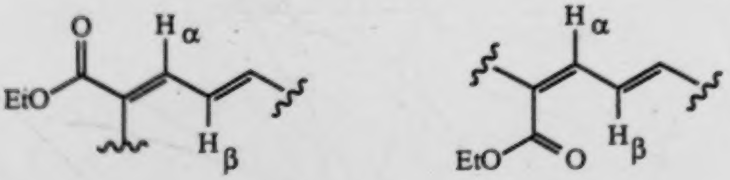
[a] In a cyclic system an all-trans isomer may not represent the most stable geometry.



A **B**

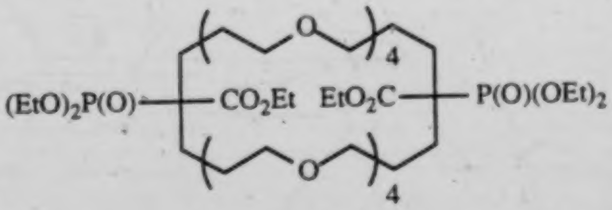
Fig. 4. Spatial disposition of hydrogen atoms α and β with respect to carboethoxy group in **70** and **71**.

One may speculate that for case **A** proton α would be deshielded more than proton β by the cis-disposed carboethoxy group and, correspondingly, for case **B**, the reverse situation would apply as here the carboethoxy group is brought close to proton β. This interpretation suggests that **70** has an all-trans geometry since its α proton is at the lower field. This interpretation was later put on a more secure basis when we prepared the sulphur analogs of **70** and **71**. The corresponding sulphur analog whose high field proton NMR spectrum in the unsaturated region was very similar to that of **70**, was found by X-ray analysis to be the all-trans isomer, in agreement with our spectroscopic conclusions.

II.7. Attempts to improve the synthesis of macrocycle 70.

Having obtained a sample of the macrocyclic heptaene polyether **70**, we decided to devote some effort to improving the route. First, we hoped to raise the yield in the alkylation step leading to diphosphonate **69**, and secondly, we wanted to design a better approach to its polyether precursor. The technique we had used involved a tedious chromatographic separation of a mixture of oligomers, so a route that would allow construction of the polyether in a controlled fashion would clearly be preferable.

Several experiments were conducted to establish the best conditions for the alkylation step leading to **69** (*cf.* Eq.18). First, we investigated the influence of counter ion on the yield. Different bases were used to generate the carbanion from triethyl phosphonoacetate, which was then alkylated with dimesylate **68** in THF (Eq 18). Sodium hydride gave the best results and yields up to 35% were obtained. Use of potassium hydride or butyllithium resulted in lower yields (25%). Next, we tried sodium hydride and 18-crown-6 but this combination did not lead to any improvement. A small amount of another product, which we had not observed before, was isolated in this experiment. Its high field ^{1}H NMR spectrum was consistent with structure **72**. It is not clear how the crown ether mediates formation of this compound, but, as the yield was very low, the matter was not pursued.



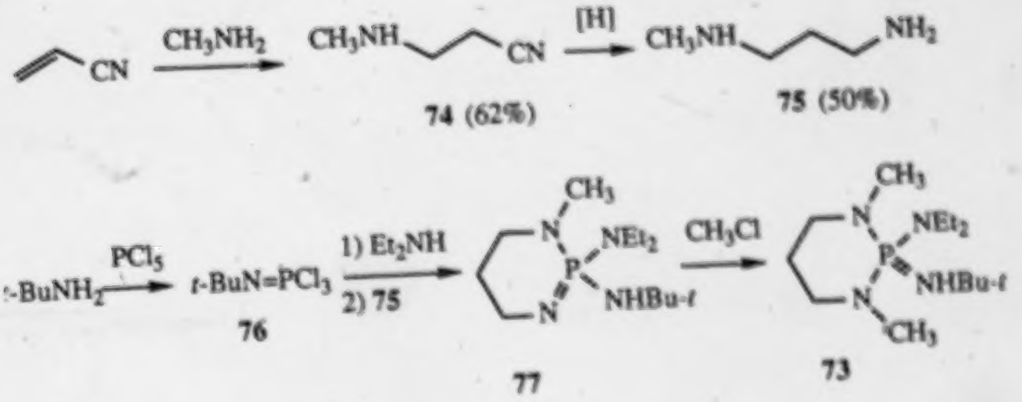
72

Increasing solvent polarity did not improve the yield of diphosphonate **69**. With acetonitrile[a] the yield was 23%. As already mentioned, we suspected that the presence of metal ions caused the polyether to wrap around the ions in a way that rendered the termini of the chain to be less accessible than in an open conformation. We wanted to conduct an experiment in the absence of metal ions. Fortunately, there are available several strong organic bases, and one of these, **73** (see Scheme 33), caught our attention since it is possible[104] to alkylate diethyl malonate using this organic base. Diethyl malonate is a substrate of similar acidity to triethyl phosphonoacetate, and so the reported experiment is an encouraging model for our purposes. We attempted to prepare **73** according to a literature procedure[104] as shown in Scheme 33.

---

[a] Acetonitrile was chosen as we did not want to use any higher boiling solvents. Use of DMSO or HMPA requires an aqueous workup which usually decreased the yield substantially due to the relatively high water solubility of **69**.

Scheme 33



Methylamine was condensed[105] with acrylonitrile to give aminonitrile **74**, which was then reduced to diamine **75**.[106] tert-Butylamine was next treated with phosphorous pentachloride to provide phosphoimine **76**. The next step involves condensation of **76** with diethylamine and then with 75. This sequence of operations affords the cyclic product 77. We were not able to reproduce the yields claimed in the literature procedure. No conditions were reported in the literature and the author declined to provide the necessary details. Condensation of **76** with diethylamine in the presence of triethylamine proceeded well, and we isolated in one case material whose high field ^{1}H NMR corresponds to structure **78**.

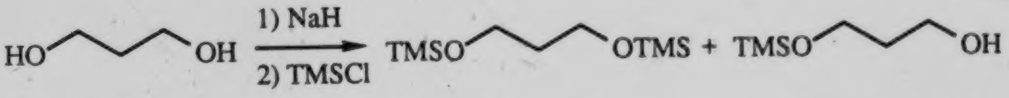
t-BuN=PCl$_2$NEt$_2$

78

However, further condensation with diamine **75** as well as final methylation gave only small amounts of impure material. Nevertheless, as the ^{1}H NMR indicated the desired structure, we attempted to alkylate triethyl phosphonoacetate **27** with dimesylate **68** in the presence of our impure sample of the organic superbase. A mixture of several products was obtained from this alkylation, and the diphosphonate **69** was present only in very low yield. Later, base **73** became commercially available and the experiment was repeated but it gave similar results. It appeared, therefore, that the conditions chosen in our early alkylations were the best ones and we decided to accept the 30-35% yield of those experiments.

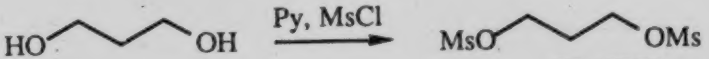
We next looked at the possibility of improving the construction of the polyether unit. Statistical condensation allows only slight changes to be made to the ratio of oligomers. We were interested in controlled assembly of the polyether by reactions that are either efficient or give easily separable products. Only such characteristics would outweigh the simplicity of the method we had already developed.

Towards this end we sought to protect selectively one hydroxyl of trimethylene diol. (We wanted to use trimethylene diol as the starting material for both components of the alkylation as trimethylene diol is fairly inexpensive.) Although a *tert*-butyldimethylsilyl group has been used for this purpose,[107] the reagent is expensive and we would need to use it on a large scale. Attempts to use trimethylsilyl chloride led to formation of a mixture of mono- and diprotected products (Eq. 20), and we were unable to separate them efficiently by vacuum distillation.



Eq. 20

However, we hoped that the diprotected diol would be inert in the subsequent alkylation step and could be distilled off at that stage. Therefore, we decided to attempt alkylation of the mixture of mono- and di-protected trimethylene diol. We needed a better leaving group than bromide and so we generated the mesylate **79** (Eq. 21).



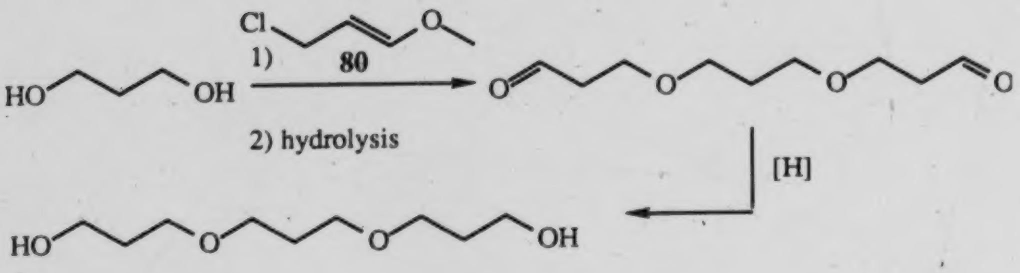
79 (60%)

Eq. 21

However, our attempts to use it to alkylate the mono-protected diol led to intractable mixtures.

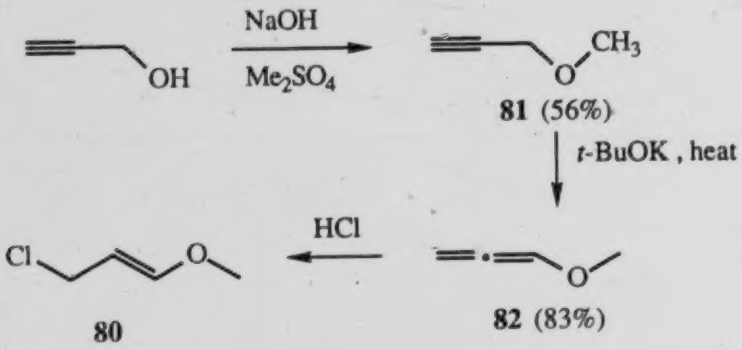
It seemed to us that use of a more active alkylating agent might be helpful so the known[108] allylic chloride **80** was chosen.

Scheme 34


Cl
O
1)
80
2) hydrolysis
HO
OH
O
O
O
O
[H]
HO
O
O
OH

In principle, the required trimer could be obtained as shown in Scheme 34 and we prepared allylic chloride **80** according to the literature procedure (Scheme 35):

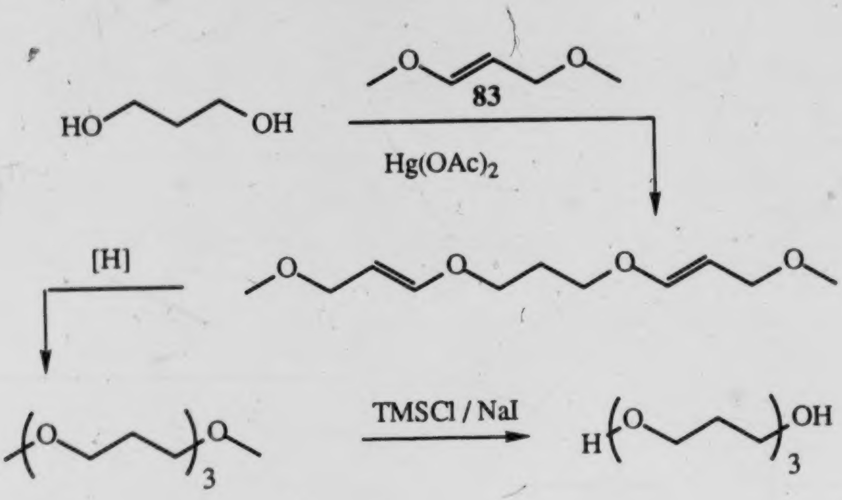
Scheme 35


NaOH
Me$_2$SO$_4$
OH
CH$_3$
O
81 (56%)
t-BuOK , heat
HCl
Cl
O
80
O
82 (83%)

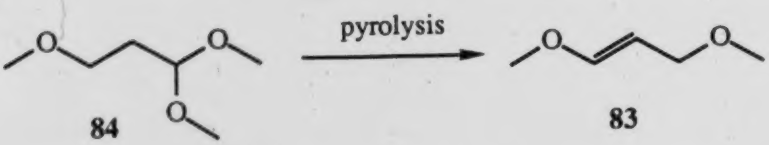
Propargyl alcohol was methylated with methyl sulfate[109] to give propargyl methyl ether **81**, which was isomerized[110] to the thermodynamically more stable allenyl methyl ether **82**. Chloride **80** was then obtained by addition of hydrogen chloride to **82**. Compound **80** is unstable; it cannot be obtained in pure form and should be used at low temperature, immediately after preparation. The high field ^1H NMR spectrum of this material indicated that the trans isomer is the major component. When we came to use chloride **80** to alkylate trimethylene diol only complex mixtures were formed.

Difficulties in alkylation reactions prompted us to explore the use of vinyl ether exchange as a means for building polyether systems as shown in Scheme 36.

Scheme 36

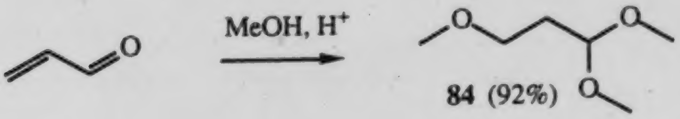


The appropriate vinyl ether **83** is a known substance[111] and it was obtained by pyrolysis of trimethoxypropane **84** (Eq. 22).



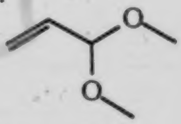
Eq. 22

We modified the literature[112] procedure for the synthesis of **84** from acrolein and methyl alcohol and obtained the desired product in good yield (Eq. 23).



Eq. 23

Isopropyl sulfate, which is used as catalyst in the pyrolysis of **84** was obtained by passing propylene through sulfuric acid. We attempted to separate the reaction mixtures from the pyrolysis by spinning band distillation, but unfortunately, the only pure material we were able to obtain in this way was acrolein acetal **85**, which is one of the products of pyrolysis.



85

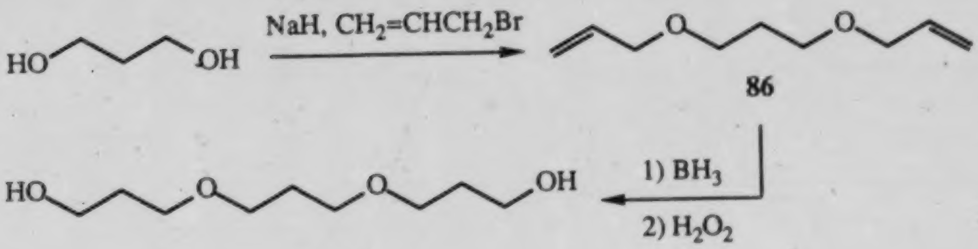
We had analyzed the distillate by V.P.C. and had noticed that during distillation, components of the mixture undergo either decomposition or interconversion. Redistillation of a fraction enriched in one component led to a more complex mixture and so we decided to abandon this path.

In the recent patent literature, there is a procedure for condensation of acrolein with alcohols in a Michael-type addition with the use of a special palladium–cadmium alloy catalyst.[113] We hoped that this process could be utilized in our case. It would, however, require reversal of the ratio of acrolein and alcohol.[a] The necessary catalyst was prepared by electrolysis of a solution of palladium dichloride and cadmium dichloride. When the addition of trimethylene diol to acrolein was attempted only complex mixtures were obtained, as shown by V.P.C. analysis.

We investigated two more processes for stepwise assembly of trimethylene polyethers.

The first route was hydroboration followed by oxidation (Scheme 37).

Scheme 37



The diallyl derivative **86** was obtained in high yield from trimethylene diol and allyl bromide, and a series of hydroboration–oxidation reactions was then carried out. The first experiment with borane–dimethyl sulfide complex showed that in our system, the hydroboration step is not completely regiospecific and results in formation of 10–15% of secondary hydroxyl groups (as judged by ^{1}H NMR measurements). Secondary boranes are known to isomerize to primary isomers at elevated temperature;[114] however, when the reaction mixture after hydroboration was heated at 160°C we could not detect any change in the level of secondary alcohols. Use of a more hindered hydroborating agent, disiamylborane prepared from 2-methyl-2-butene[115] lowered the content of secondary alcohols slightly. Perhaps, even more regiospecific reagents would afford only primary diols, but such reagents would be prohibitively expensive for large scale work.

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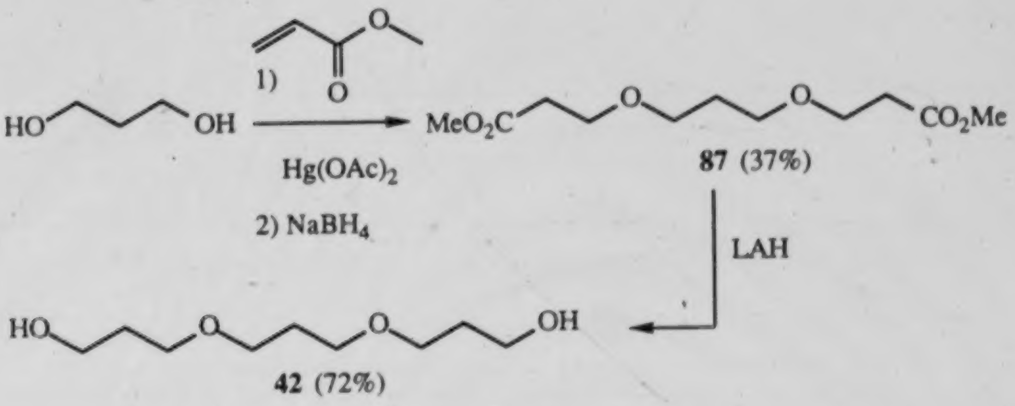
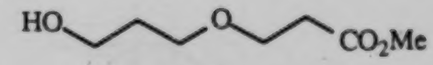
[a] The reported results were obtained with a large excess of alcohol, whereas for our purposes an excess of acrolein was needed.

The last route we examined is summarized in Scheme 38.

Scheme 38



Reaction of trimethylene diol with methyl acrylate, used in large excess (300%), and mercuric acetate (some 10-20% excess) followed by reduction with sodium borohydride, led to formation of a mixture of **87** and **88**.



88

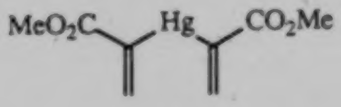
We were unable to drive the reaction to completion by increasing the amounts of methyl acrylate or mercuric acetate. However, **87** and **88** can be easily separated by taking advantage of the greater water solubility of **88**. Extraction of an ethereal solution of the mixture with water, removes **88** completely[a] and vacuum distillation then affords pure **87**. Moreover, in principle, the recovered **88** can be recycled to increase the overall yield. Reduction of **87** with lithium aluminum hydride proceeds smoothly[b] to afford pure **42**. Repetition of the sequence should give the pentameric analog and although use of chromatography may be necessary (we did not try this experiment) the separation would be much easier than in the case of separating the mixture of benzoates.

While searching for conditions that would result in lowering the amounts of **88**, we discovered that when the reaction is conducted in the presence of triethylamine, the alkoxymercuration with trimethylene diol does not occur at all. Instead, we isolated material which was identified as mercuride **89**.

---

[a] An alternative solution is to use another mercury salt. Perchlorate is a good choice because of much lower nucleophilicity of perchlorate anion compared to acetate, but we did not try it.

[b] Aqueous workup leads to low yield due to high solubility of the trimeric diol in water (unless an efficient continuous extraction technique is used); by destroying the excess of lithium aluminum hydride with ethyl acetate and neutralizing the reaction mixture with concentrated hydrochloric acid one can recover ca 80% of product.



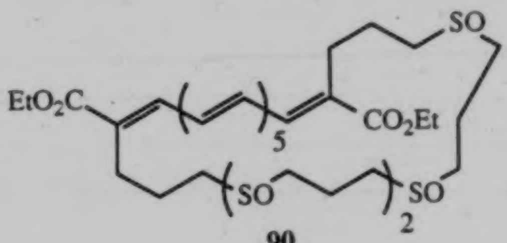
89

We did not pursue this observation; however, it seems that if the phenomenon is of a general nature, it may be a convenient way of functionalizing α,β-unsaturated carboxylic esters on an α carbon.

At this point we learned that the model macrolide **70** did not show antifungal activity.[116] Accordingly, we had to revise our synthetic plan to modify the hydrophilic unit of the molecule.

II.8. Synthesis of sulphur analog of 70.

Because biological tests on the polyether macrolide **70** showed the substance to be inactive, we decided to prepare the corresponding polysulfoxide macrocycle **90**.

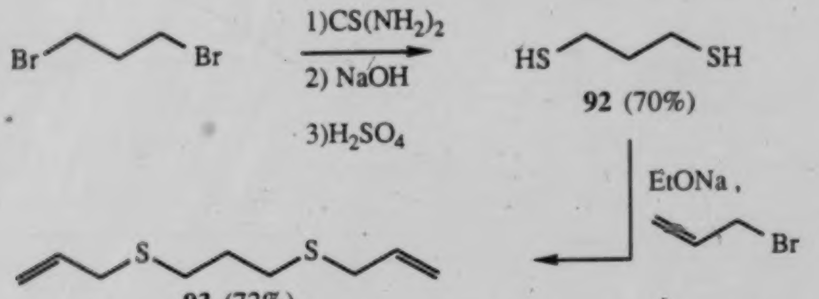


90

Such a compound would have greater water solubility from the polyether **70** because of the polar nature of the sulfoxide units. We had found that the polyether **70** was not water-soluble and this property may have prevented it from operating as an antifungal agent. The presence of sulfoxide groups would also provide a mechanism for self-assembly of the individual molecules by dipole-dipole interaction[117]—a characteristic present, although by a different mechanism, in Amphotericin B itself, but absent from the polyether **70**. A natural choice for the precursor to the new model was the sulfur analog of **70** wherein all ethereal oxygens are replaced by sulfur atoms. We felt that direct assembly of a polysulfoxide unit might pose some practical problems due to extreme polarity and, therefore, we decided to make a sulphur analog of **70** first, and to oxidize the heteroatoms at a later stage. Additionally, formation of polythioether would provide an opportunity to examine the possibility of *in vivo* oxidation to the polysulfoxide level so that the polythioether would act in the manner of a prodrug. Some microorganisms are capable of oxidizing sulfides to sulfoxides.[118]

Our synthetic plan was closely related to what we had already done and was based on the diphosphonate **91** (see Scheme 40). In this case, however, it was possible to overcome some of the problems encountered earlier in our attempts at a controlled oligomerization of oxygen-containing starting materials. We used a combination of alkylation on sulfur and free radical addition of thiol units to double bonds as a means of forming the required polysulfide, as shown in Scheme 39, 40 and 41 and Eq. 24.

Scheme 39

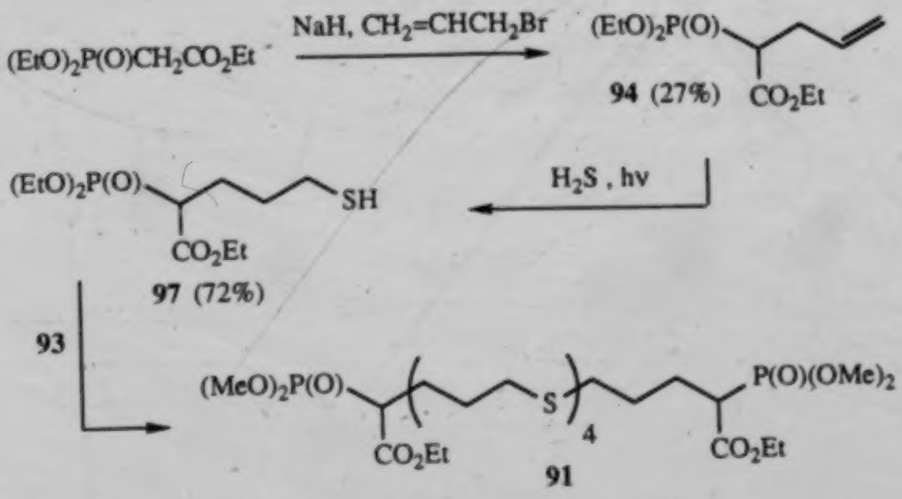


92 (70%)

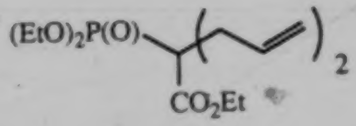
93 (72%)

1,3-Dibromopropane was converted (Scheme 39) into the 1,3-dithiol **92** by a standard route using thiourea, and the resulting dithiol **92** was alkylated with allyl bromide. These two steps afforded the diallyl bis-sulfide **93**.

Scheme 40

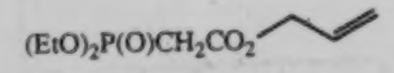


For the other component needed in the assembly of our polysulfide we next carried out an alkylation of triethyl phosphonoacetate with allyl bromide (Scheme 40). This reaction did not proceed smoothly. Apart from the desired material **94**, a doubly alkylated derivative **95** was formed as well.



95

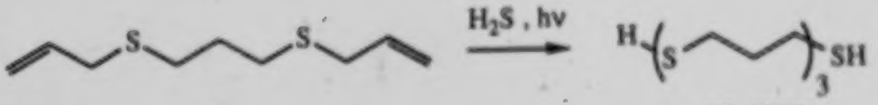
As the separation of **94** and **95** by spinning band distillation was tedious, we tried to find better conditions for preparation of **94**. However, even when a solution of the sodium salt of triethyl phosphonoacetate in tetrahydrofuran was added slowly to a threefold excess of allyl bromide, we still observed considerable amounts of doubly alkylated product **78**. Running the reaction in ethanol gave poorer results, and probably led to some *O*-alkylation (as judged by the isolation of allyl ester **96**).



96

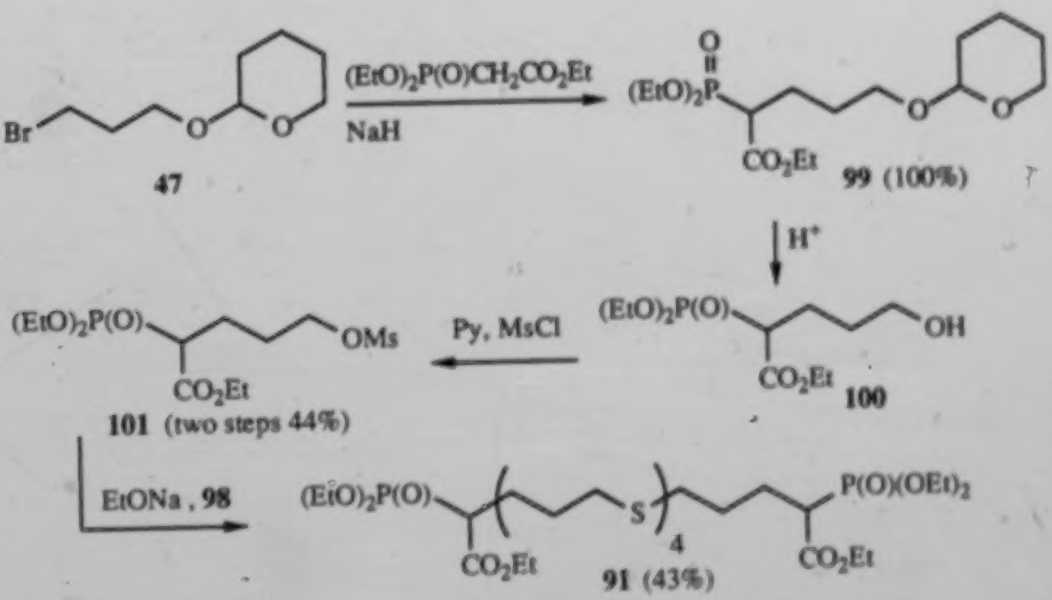
It seems that it is difficult to avoid the second alkylation completely, but the extent can be minimized. Alkylation should be done without solvent and with potassium carbonate as base. These conditions have been found to be very effective in such cases.[119] We conducted an experiment along these lines and monitored the progress of the reaction by V.P.C. Although a considerable amount of dialkylated product is present when the reaction is close to completion, the ratio of di- to monoalkylated material is quite low at earlier stages of the reaction. It would appear that the reaction should be stopped just before this ratio exceeds an acceptable level (although we did not actually do this). The allyl derivative **95** was then converted into the requisite mercaptan **97** by photochemical addition of hydrogen sulfide. The last step of Scheme 40 (i.e., reaction of **97** and **93** also under photochemical conditions) resulted only in very small quantities of impure diphosphonate **91**. The alternative route shown in Scheme 41 was tried, and it proved satisfactory.

The necessary dithiol **98** was obtained by photochemical addition of hydrogen sulfide to diallyl bis-sulfide **93** (Eq. 24).


H_2S , hv
H
S
SH
3

93 98 (28%) Eq. 24

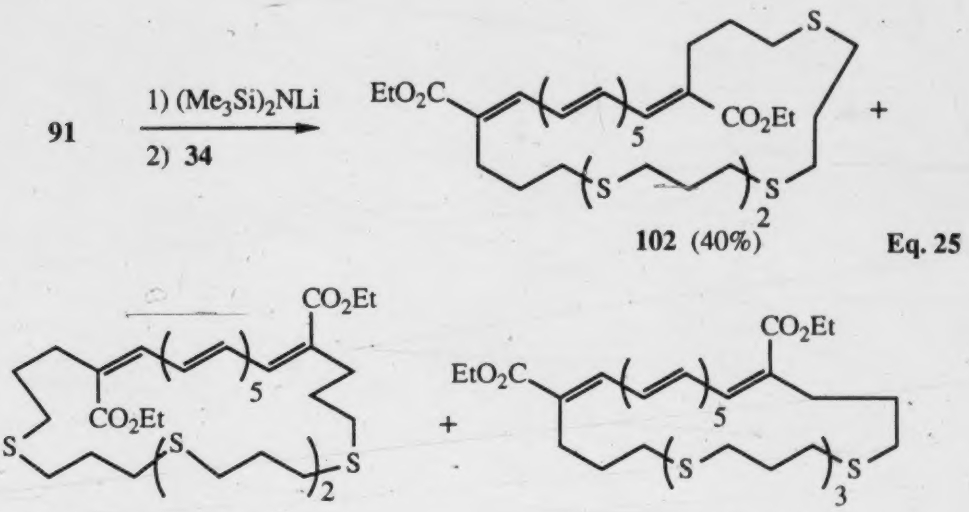
Scheme 41


Br
O
O
$(EtO)_2P(O)CH_2CO_2Et$
NaH
O
$(EtO)_2P$
CO_2Et
H^+
$(EtO)_2P(O)$
OH
CO_2Et
Py, MsCl
$(EtO)_2P(O)$
OMs
CO_2Et
EtONa , 98
$(EtO)_2P(O)$
S
4
$P(O)(OEt)_2$
CO_2Et
CO_2Et

47 99 (100%) 100 101 (two steps 44%) 91 (43%)

Triethyl phosphonoacetate was alkylated with compound **47** to afford **99**. The tetrahydropyranyl protecting group was removed, and the alcohol **100**, obtained in this way, was then mesylated to produce **101**. Later, on repeating the experiment, we decided not to isolate **100** and to carry out the mesylation on the crude alcohol **100**. Mesylate **101** was used to alkylate dithiol **98**, affording the desired diphosphonate **91**.

We were now in a position to construct the macrocycle, and as expected, cyclization of **91** with dialdehyde **34** (Eq. 25) proceeded smoothly.



102 (40%)

Eq. 25

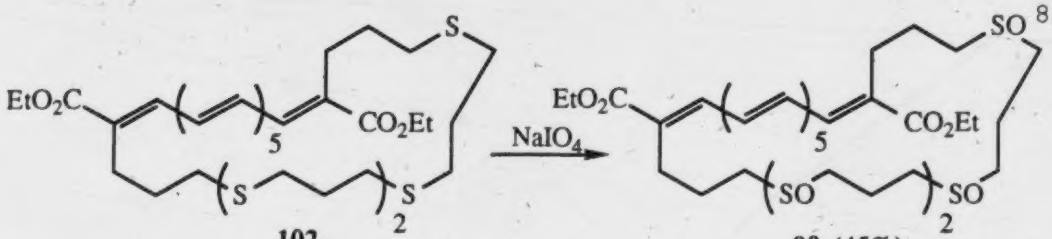
103 (0.2%)

104 (6%)

We obtained macrocycle **102** as well as two isomers, **103** and **104**. Compound **103** was formed in very low yield; however, it was possible to measure its ^1H NMR spectrum. One diagnostic set of signals was clearly discernable: The quartet corresponding to the methylene protons of the carboethoxy group came up very cleanly, indicating that the terminal double bonds of the heptaene system are of the same geometry. Isomer **104**, similarly to its oxygen analog, was obtained in impure form and was unstable. As in the corresponding oxygen series (i.e., compound **71**) it was possible to isomerize **104** to **102** by the catalytic action of iodine in dichloromethane. In the ^{1}H NMR spectrum of **104** the signals corresponding to the allyl protons are hidden in multiplets arising from protons on carbons α to sulfur atoms. However, the methylene protons of the carboethoxy groups give rise to a multiplet, which probably is a result of overlapping of two quartets. This multiplicity indicates that the terminal double bonds of the heptaene system are of different geometry. Analysis of the unsaturated region fully supports this statement. The signals corresponding to protons α and β (see Fig.4 page 67) are a more complex, but understandable, pattern. In the low field region there is a multiplet (2 H), apparently a result of overlapping of a quartet and a doublet, in the higher field region, a quartet and a doublet (each 1 H). These patterns lead to the conclusion that both systems A and B (see page 67), are present in the molecule of **104**.

The high field ^{1}H NMR spectrum of isomer **102** was compared with the spectrum of **70**. In both spectra the signals corresponding to protons from the heptaene unit are very similar in appearance. This indicates that in both substances the geometry of the polyene system is identical. Nevertheless, except for the arguments already presented we did not have rigorous proof for the structure that we assign to **102**. Therefore we decided to obtain a crystal structure of this material. Some problems were encountered in growing crystals of appropriate size, but it was possible to obtain samples of sufficient quality for a partial analysis. The results of the X-ray determination confirm that the polyene unit is indeed in an all-trans arrangement (see Appendix C). The crystal was highly disordered, and it was not possible to determine the exact positions of the atoms constituting the middle part of polysulfide chain.

At this point we examined ways of obtaining the corresponding polysulfoxide. One solution was to oxidize the diphosphonate **91** and carry out the cyclization as before. As mentioned above, a more attractive possibility was direct oxidation of **102**. We tried both approaches. Oxidation of phosphonate **91** with *m*-chloroperbenzoic acid gave material that was so polar that we felt that separation of isomers of the cyclized material would be impossible. Therefore we oxidized the macrocycle **102**. Obviously, we could not use peracid for this purpose, but sodium periodate appeared suitable. The reagent is normally employed in an aqueous medium but our material **102** is not water soluble. Fortunately, there is a way to circumvent the problem of solubility and that involves use of reagents on an appropriate support. Sodium periodate may be deposited on alumina, and in this form it has been used to oxidize sulfides to sulfoxides in alcoholic solution.[120] We prepared the supported reagent and attempted an oxidation of **102** in ethanol (Eq. 26).



102 **90** (45%)

Eq. 26

The reaction was fairly slow and several products were formed. We noticed that an excessive reaction time results in decomposition of the initial products, and undoubtedly involves formation of sulfones. We repeated the reaction under slightly different conditions. To increase the solubility of the substrate in the reaction medium, we used a mixture of tetrahydrofuran and methanol as solvent and we monitored the progress of the reaction closely by TLC (silica, methanol). We assumed that the most polar component of the mixture would be the requisite tetrasulfoxide **90** and that less polar components formed later would correspond to products containing one or more sulfone groups. With those assumptions in mind we stopped the reaction when formation of a new product of relatively low polarity was detected. Two fractions were separated and mass spectrometry confirmed our reasoning. The most polar component was indeed the tetrasulfoxide and the molecular weight of the less polar one corresponded to a mixture of partially overoxidized materials. We measured the ^{1}H and ^{13}C NMR spectra of **90** and they confirm its structure.

It is not clear what role, if any, the alumina support plays in this reaction; however, we noticed two interesting facts. The reagent is active only for a short period after drying in an oven for at least 24 h at 120°C. Stored for a few days in desiccator it loses most of its ability to oxidize **102** and the reaction is extremely slow. Secondly, during an experiment performed in methanol (which slightly dissolves sodium periodate), we observed that failure to wash the reaction mixture with water (upon addition of dichloromethane) prior the evaporation, results in increased amount of sulfones but not of **90**.

X-ray analysis of **102**, apart from telling us the geometry of the polyene unit, revealed that the polysulfide unit is more than sufficiently long to span the ends of the polyene (Fig. 5) and we were concerned that this may hinder the formation of channels.

Comparison of a simple, computer model[a] of **102** (Fig. 6) with its X-Ray structure (Fig. 5), shows, that this type of model is a fair approximation of the gross shape of the molecule and may be used for our purpose. We decided to synthesize another macrocycle with a shorter polysulfide chain. Removal of one trimethylene sulfide unit from the polysulfide chain of **102** appeared to be appropriate (Fig. 7) and we set out to make macrocycle **105** by the route shown in Scheme 42.

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[a] The calculation were performed on a Macintosh II computer with the use of the Chem3D program, courtesy of Dr. J. Vederas of this Department.

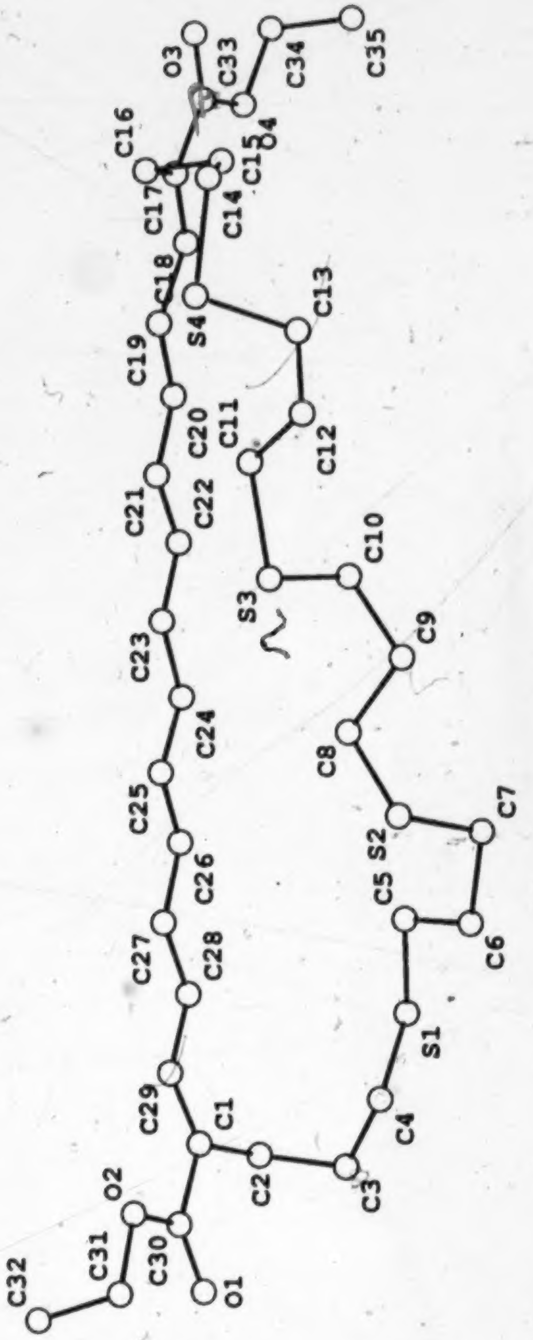

C35
C34
C33
O3
O4
C16
C15
C17
C14
C18
C13
S4
C19
C11
C12
C20
C10
C21
C22
S3
C9
C23
C24
C8
C7
C25
S2
C5
C26
C6
C27
C28
S1
C29
C1
C4
O2
C2
C3
C31
C30
C32
O1

Fig. 5. X-Ray structure of **102**.

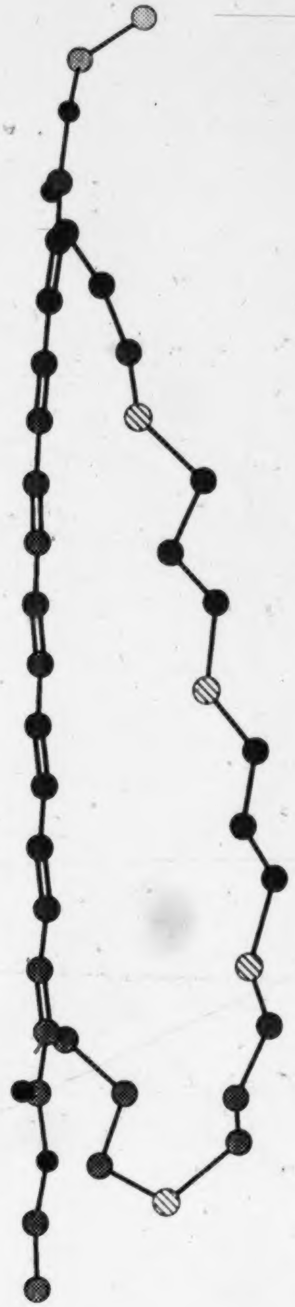


Fig. 6. Molecular model of **102**.

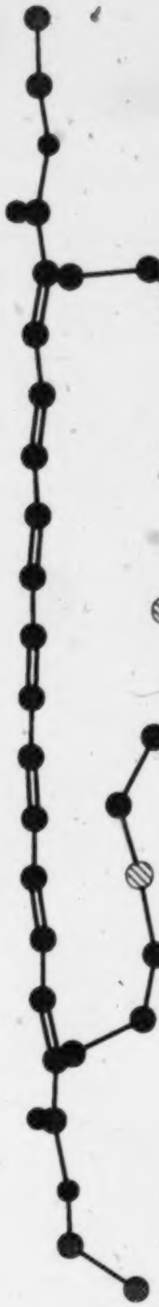
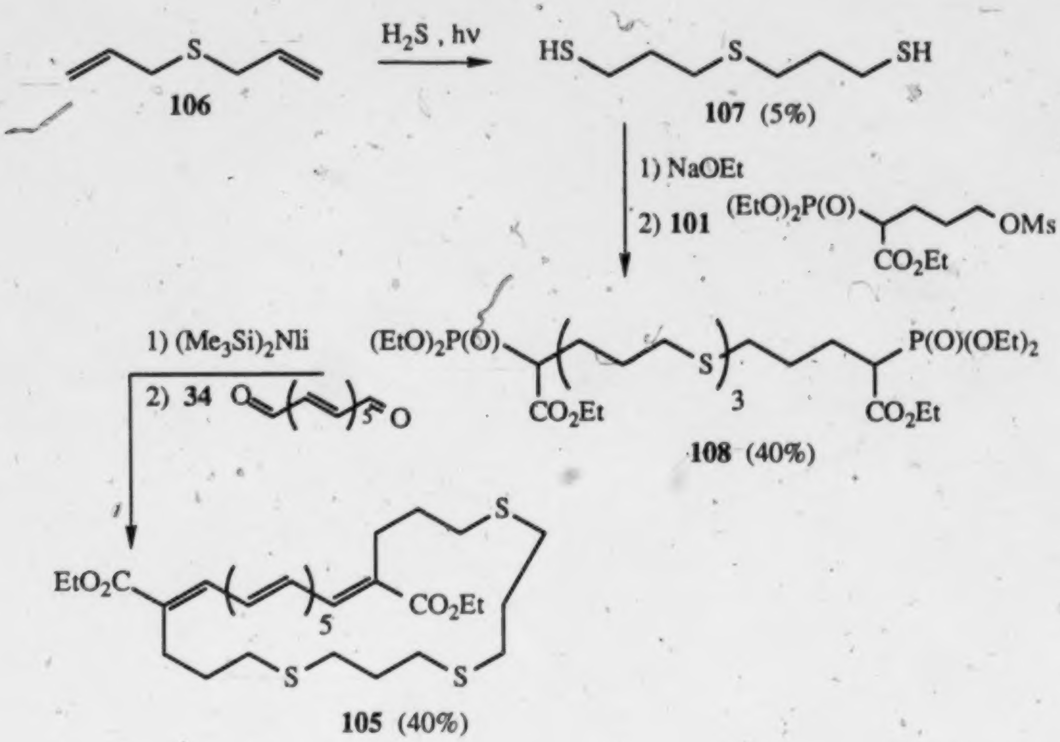
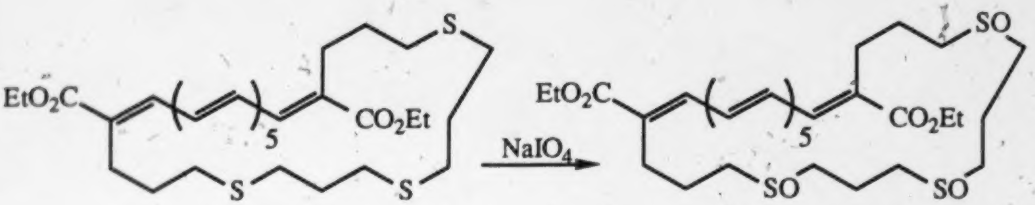


Fig. 7. Molecular model of **105**.

Scheme 42



Photochemical addition of hydrogen sulfide to diallyl sulfide **106** afforded dithiol **107**. Next, dithiol **107** was alkylated with mesylate **101** to give diphosphonate **108**. Finally cyclization of **108** with dialdehyde **34** under the conditions used previously for the synthesis of **70** and **102** afforded cyclic material **105**. This product was accompanied by very small quantities of other materials that are possibly its geometrical isomers. The analysis of the ^1H high field NMR spectrum of **105** revealed that it has the same geometry of the heptaene unit as **70** and **102**, and we assign the all-trans structure.



105

109 (29%)

Eq. 27

Next we attempted to oxidize **105** (Eq. 27). We used the same conditions as previously with **102**, and although in this case the reaction was slower, we isolated material whose molecular mass corresponds to that of **109**. The ^{1}H spectrum of **109** is consistent with the proposed structure.

The compounds **90**, **102**, **105** and **109** were subjected to biological tests,[116] but disappointingly, none of them showed antifungal activity towards yeasts.

III. CONCLUSION

The results presented in the previous chapter show that a double Wittig reaction may be succesfully used to obtain macrocycles with a ring size corresponding to that of large polyene antibiotics.

It is not clear whether the lack of biological activity of the compounds that were synthesized (particularly the polysulfoxides **90** and **109** which are extremely polar materials) is a result of an inability of the model compounds to self-assemble or is due to a lack of some other structural feature absent from the models but present in the natural materials.

In order to decide this matter, an analog of AmB which is equipped with hydroxyl groups is needed. Such a material would possess all the basic features of the polyene antibiotics (except for the polar moiety which, however, is missing from some members of this group, for example, Filipin **8**). Work towards the synthesis of such a compound has been started and the results are described in Appendix A.

IV. EXPERIMENTAL

General Procedures: Except where stated to the contrary, the following particulars apply: All experiments were done under a slight static pressure of argon, purified by passage through a column (3.5 x 42 cm) of R-311 catalyst[121] and then through a similar column of Drierite. Glassware was dried in an oven for at least 3 h before use (120°C) and either cooled in a desiccator over Drierite, or assembled quickly, sealed with rubber septa, and allowed to cool under a slight static pressure of argon. Reaction mixtures were stirred by Teflon-coated magnetic stirring bars. When solutions were added over a specified time and a rinse was used to wash all the material into the reaction vessel, then the time stated refers to the main solution; the rinse was usually added at a fast dropwise rate.

Solvents for chromatography or extractions were distilled before use. Where required, solvents for reactions were dried by distillation from a suitable drying agent (see below) under argon, and were transferred by oven-dried glass syringes or by sterilized disposable syringes (Aldrich).

Products were isolated from solution by evaporation under water-pump vacuum at 25°C using a rotary evaporator. In those cases where compounds were isolated simply by evaporation of their solutions (and not also by subsequent distillation) the residues were kept under oil-pump vacuum (0.1 mm) and checked for constancy of weight. Isolated products were submitted directly for combustion analysis, without need for further purification. Melting points were determined on a Kofler block melting point apparatus. Boiling points reported for products distilled in a Kugelrohr apparatus refer to the oven temperature.

Perkin Elmer model 151 or 251 annular stills were used for spinning band distillations.

Commercial thin layer chromatography (TLC) plates (silica gel, Merck 60F-254) were used. Spots were detected by examination under UV light followed by exposure to iodine, by spraying the plate with 6 N sulfuric acid in methanol, with 6 N sulfuric acid in methanol containing 0.1 mL of anisaldehyde in 100 mL of the solution, or with 5-10% w/v solution of phosphomolybdic acid in 6 N methanolic sulfuric acid, followed by charring on a hot plate. Silica gel for flash chromatography was Merck type 60 (230-400 mesh).

All vapor phase chromatographic (V.P.C.) analyses were performed on a Hewlett-Packard 5830A gas chromatograph equipped with an FID detector using pre-packed Hewlett-Packard 6 ft x 1/8 in o.d. stainless steel analytical columns, with nitrogen as the carrier gas. The columns used for vapor-phase chromatographic analyses were: 10% Apiezon L, 2% potassium hydroxide on acid-washed Chromosorb W (80-100 mesh); 10% QF1 on acid-washed Chromosorb W (80-100 mesh) that had been treated with dimethylchlorosilane; 10% OV-1 on high performance Chromosorb W (80-100 mesh).

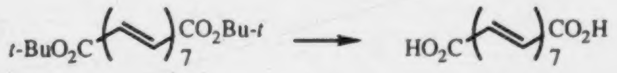
Infrared spectra were recorded on a Nicolet 7000 FT-IR spectrometer. The measurements were made as casts from the specified solvent or on Nujol mulls. Only diagnostically significant peaks are reported.

Proton nuclear magnetic resonance spectra were recorded with Bruker WP-80 (at 80 MHz), Bruker WH-200 (at 200 MHz), Bruker AM-300 (at 300 MHz) or Bruker AM-400 (at 400 MHz) spectrometers in the specified deuterated solvents using as an internal standard tetramethylsilane or the deuterated solvent residual signals. The following abbreviations are used for ^{1}H NMR spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. ^{13}C NMR spectra were recorded with Bruker WH-200 (at 50.3 MHz), Bruker AM-300 (at 75.5 MHz), and Bruker WH-400 (at 100.6 MHz) spectrometers using the specified deuterated solvent as an internal standard. High resolution mass spectra were recorded with an AEI/Kratos MS50 spectrometer at an ionizing voltage of 70 eV at 10000 resolution. Low resolution mass spectra were recorded with an AEI/Kratos MS12 spectrometer (chemical ionization) with use of ammonia or an AEI/Kratos MS9 spectrometer (fast atom bombardment) with use of Cleland's reagent (5:1 mixture of dl-dithiothreitol and dithioerythritol from Sigma)matrix and a Xenon saddle field gun at 7 kV.

Microanalyses were performed by the microanalytical laboratory of this Department.

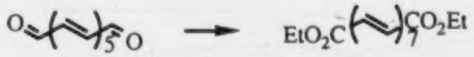
Materials: Dry solvents were prepared under an inert atmosphere. Dry benzene, ether, dioxane, and tetrahydrofuran (THF) were distilled shortly before use from sodium and benzophenone ketyl. Dry acetonitrile, diisopropylamine, triethylamine, chloroform, dichloromethane, pyridine, chlorotrimethylsilane, *tert*-butyl alcohol, and toluene were distilled shortly before use from calcium hydride. Dry hexamethylphosphoric triamide (HMPA) and dimethylformamide (DMF) were distilled from calcium hydride under reduced pressure (0.1 mm and 15 mm, respectively). Acetone was dried by distillation from anhydrous potassium carbonate. Petroleum ether refers to the fraction boiling at 30°C–60°C. Commercial (Aldrich) solutions of *n*-butyllithium in hexanes were titrated before use by the diphenylacetic acid method.[122] Lithium bis(trimethylsilyl)amide was purchased (Aldrich) as 1 M solutions in THF and was used at the stated concentration.

(*E,E,E,E,E,E,E*)-2,4,6,8,10,12,14-hexadecaheptaenedioic acid (11).



p-Toluenesulfonic acid (1 mg, 0.005 mmol) was added to a solution of **41** (10.8 mg, 0.028 mmol) in benzene (10 mL) and the mixture was refluxed for 0.5 h. During that time the deep yellow color of the solution faded and a yellow deposit was formed. The precipitate was filtered off, washed with benzene (3 mL), and dried to give **11** (7.1 mg, 93%): FT-IR (Nujol) 2953, 2923, 2854, 1699, 1620, 1591, 1461, 1376, 1308, 1244, 1166, 1138, 1015 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 5.90 (d, J = 15.0 Hz, 2 H), 6.42–6.65 (m, 8 H), 6.79 (dd, J = 14.5, 10.5 Hz, 2 H), 7.23 (dd, J = 15.0, 11.5 Hz, 2 H), 12.20 (bs, 2 H).

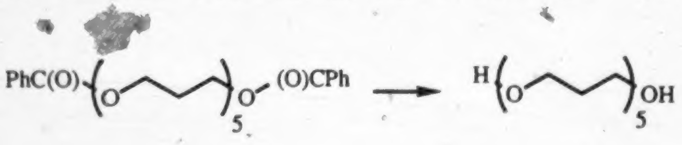
(*E,E,E,E,E,E,E*)-Diethyl 2,4,6,8,10,12,14-hexadecaheptaenedioate (16).



A solution of triethyl phosphonoacetate (4.48 g, 20.0 mmol) in THF (30 mL) was added dropwise over 3 min to a magnetically stirred and cooled (ice-bath) mixture of sodium hydride [(57% w/w suspension in mineral oil, 840 mg, 20.0 mmol) that had been washed with dry THF (3 x 2 mL)] and THF (5 mL). The cold-bath was removed and stirring was continued until a clear solution formed. A solution of dialdehyde **34** (1.184 g, 6.29 mmol) in dry THF (80 mL) was added dropwise over 20 min and the mixture was stirred overnight. The resulting precipitate was separated by centrifugation, washed with THF (3 x 5 mL; centrifugation), and dried to give pure (TLC, silica, 9:9:2 chloroform–hexane–ethyl acetate) diester **16** as brown-orange crystals (1.708 g, 82%): mp 225–226°C (from toluene) [lit.[53] mp 217°C]; FT-IR (CH_2Cl_2 cast) 2960, 1702, 1306, 1259, 1155, 1014 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (t, J = 7.0 Hz, 6 H), 4.21 (q, J = 7.0 Hz, 4 H), 5.89

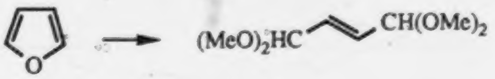
(d, J = 15.0 Hz, 2 H), 6.32–6.50 (m, 8 H), 6.60 (dd, J = 14.5, 10.5 Hz, 2 H), 7.31 (dd, J = 15.0, 11.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.37, 60.36, 121.19, 130.88, 133.39, 134.93, 136.82, 140.38, 144.17, 167.11; exact mass *m/z* calcd for $C_{20}H_{24}O_4$ 328.1674, found 328.1671.

4,8,12,16-Tetraoxanonadecan-1,19-diol (22).



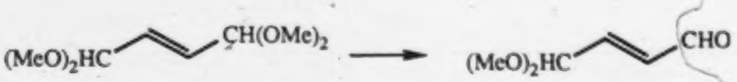
A solution of potassium hydroxide in methanol (1.74 M, 0.24 mL, 0.416 mmol) was added to a magnetically stirred solution of pentamer dibenzoate **51** (108 mg, 0.208 mmol) in ether (20 mL) and stirring was continued until all the ester was hydrolyzed (TLC, 1:3 ethyl acetate–chloroform). A solution of sulfuric acid (0.65 M, 1 mL) was added followed by triethylamine (0.02 mL) (to ensure neutral conditions) and the precipitate was removed by centrifugation. The solid was washed with ether (3 x 5 mL) and the combined organic solutions were evaporated. Flash chromatography of the residue over silica gel (2 x 13 cm) with 3:7 acetone–ethyl acetate gave pure (V.P.C.) pentameric diol **22** (55 mg, 86%): ^1H NMR ($CDCl_3$, 300 MHz) δ 1.78–1.88 (m, 10 H), 2.88 (bt, J = 5.0 Hz, 2 H), 3.46–3.55 (m, 12 H), 3.61 (t, J = 4.0 Hz, 4 H), 3.75 (q, J = 5.0 Hz, 4 H). The substance was characterized as its dibenzoate (see later).

(*E*)-1,1,4,4-Tetramethoxybut-2-ene (25).[90]



A solution of bromine (64.2 g, 0.40 mol) in methanol (160 mL) was cooled to -78°C, transferred in portions to an addition funnel, and added dropwise over 2 h with mechanical stirring and cooling [-45°C (±5°C)] to a solution of freshly distilled furan (27.7 g, 40.1 mol) in methanol (160 mL). The temperature of the reaction mixture was raised to -15°C, by intermittent removal of the cold-bath, and stirring was continued for 1 h. The mixture was cooled again to -50°C and ammonia was passed through the solution until pH 8 was reached. During this time the temperature was maintained at -40°C, a copious precipitate was produced, the slurry became thick, and manual shaking was required. The cold-bath was removed, and when the mixture had attained room temperature (*ca* 30 min), some additional ammonia was passed in (to pH 9). The precipitate was filtered off and washed with ether (2 x 100 mL). The filtrates were combined and the solvents were evaporated. The residue was distilled under reduced pressure to give **25** (40.8 g, 58%) as a colorless liquid: bp 74°C (4.5 mm) [lit.[90] 83%, bp 88–90°C (13 mm)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.31 (s, 12 H), 4.83 (dd, J = 2.0, 1.0 Hz, 2 H), 5.83 (dd, J = 2.0, 1.0 Hz, 2 H).

(*E*)-4,4-Dimethoxybut-2-enal (26).[123]



Phosphoric acid (6% w/v solution, 11.24 mL, 0.58 mol water) was added with magnetic stirring to diketal **25** (100 g, 0.57 mol) and the mixture was stirred and heated on a water bath until no more methanol distilled over. Calcium carbonate powder (5 g) was added and stirring was continued at room temperature for 30 min. The mixture was filtered and the filtrate was distilled under reduced pressure to give the crude product in several fractions of total weight 68 g: bp 57–72°C (3.2 mm). Redistillation using a spinning band apparatus [Perkin-Elmer 251 Auto Annular Still] afforded **26** (53.8 g, 72 %) as a pale yellow oil: bp 69–70°C (3.1 mm) [lit.[123] 67%, bp 72–80 °C (11 mm)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.39 (s, 6 H), 5.10 (dd, J = 3.8, 1.1 Hz, 1 H), 6.38 (ddd, J = 16.0, 8.0, 1.1 Hz, 1 H), 6.70 (dd, J = 16.0, 3.8 Hz, 1 H), 9.65 (d, J = 8.0 Hz, 1 H).

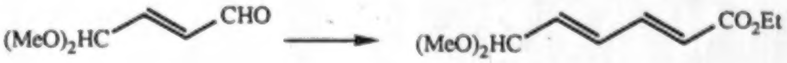
Triethyl phosphonoacetate (27).[124]

$$BrCH_2CO_2Et \longrightarrow (EtO)_2P(O)CH_2CO_2Et$$

Ethyl bromoacetate (70.0 g, 0.42 mol) was added dropwise to a magnetically stirred and heated (oil bath, 100°C) portion of triethyl phosphite (70.0 g, 0.42 mol). The ethyl bromide that distilled over was collected and the rate of addition was controlled so as to maintain moderate boiling. When the addition was complete the bath temperature was raised to

150°C and stirring and heating was continued for 3 h. At that point the weight of ethyl bromide collected corresponded to 80% conversion. The reaction mixture was distilled under reduced pressure to afforded **27** as a colorless liquid (78.3 g, 83%): bp 101°C (0.5 mm) [lit.[124] 88%, bp 109–109.5 °C (0.8 mm)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.07–1.24 (m, 9 H), 2.80 (d, J = 22.0 Hz, 2 H), 3.93–4.11 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 13.80, 16.05, 33.52, 34.86, 61.16, 62.38, 165.49.

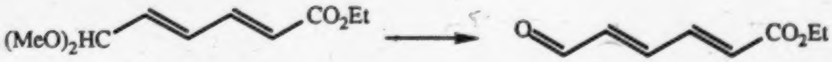
(*E,E*)-Ethyl 6-dimethoxy-2,4-hexadienoate (28).[87]



Triethyl phosphonoacetate **27** (22.4 g, 0.100 mmol) was added dropwise over 30 min to a magnetically stirred and cooled (ice-bath) mixture of sodium hydride [(57% w/w suspension in mineral oil, 4.30 g, 0.102 mmol) that had been washed with dry benzene (3 x 2 mL)] and benzene (30 mL). The rate of addition was controlled so that the temperature remained below 30°C. The cold-bath was removed and stirring was continued for 1 h. Dimethoxycrotonaldehyde **26** (13.0 g, 0.100 mmol) was added dropwise over 45 min and stirring was continued overnight. The mixture was warmed to 60°C, stirred for 15 min at this temperature, cooled, poured onto ice (100 g), and extracted with ether (3 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated, and the residue was distilled to give **28** as a colorless oil (12.10 g,

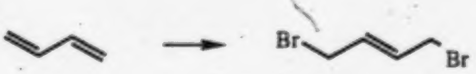
60.4%): bp 90°C (0.40 mm) [lit.[87] 54%, bp 94°C (0.05 mm)]; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 1.30 (t, J = 7.0 Hz, 3 H), 3.32 (s, 6 H), 4.20 (q, J = 7.0 Hz, 2 H), 4.80 (d, J = 4.0 Hz, 1 H), 5.85–6.15 (m, 2 H), 6.31–6.70 (m, 1 H), 7.29 (dd, J = 15.0, 11.0 Hz, 1 H).

(*E,E*)-Ethyl 6-oxo-2,4-hexadienoate (29).[86,87]



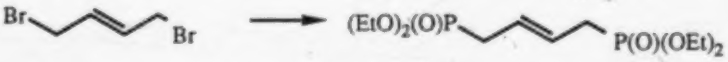
A mixture of **28** (19.17 g, 95.7 mmol), acetic acid (93 mL), sodium acetate (9.3 g), and water (6.2 mL) was stirred and heated on a water bath. The progress of the reaction was monitored by TLC (silica, 7:1 hexane–ethyl acetate). When the hydrolysis was complete (*ca* 1.5 h), the mixture was poured onto ice (400 g) and extracted with ether (4 x 100 mL). The combined organic extracts were stirred with saturated aqueous sodium bicarbonate (100 mL) until no further evolution of carbon dioxide was observed. The organic phase was dried ($MgSO_4$) and evaporated. Distillation of the oily residue gave aldehyde **29** as pale yellow oil which solidified on storage (12.14 g, 82%): bp 62–64°C (0.07 mm) [lit.[87] 81%, bp 100°C (1 mm), lit.[86] 80%, bp 76–80°C (0.4 mm)]; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 1.32 (t, J = 7.0 Hz, 3 H), 4.26 (q, J = 7.0 Hz, 2 H), 6.20–7.64 (m, 4 H), 9.69 (d, J = 7.0 Hz, 1 H).

(*E*)-1,4-Dibromobut-2-ene (31).[125]



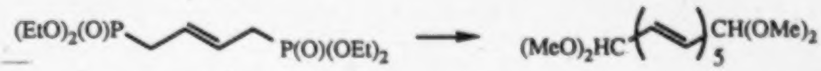
Bromine (160 g, 1 mol) was added with mechanical stirring to a cooled solution of butadiene (54 g, 1 mol) in hexane (150 mL) at such a rate that the temperature was maintained at -15 (±5)°C. The mixture was cooled to -30°C and the precipitate that formed was filtered off quickly and then crystalized from hexane to give **31** (62 g, 29%). Additional product was obtained from the combined mother liquors. These had been stored, without any protection from light, and, after a period of about 2 months, the precipitate was collected and crystalized from hexane to give more **31** (45 g). The total yield of product corresponds to 50% and the material had: mp 49–51°C [lit.[125] mp 54°C]; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.91–3.94 (m, 4 H), 5.93–5.97 (m, 2 H).

Tetraethyl but-2-ene-1,4-diylbisphosponate (32).[126]



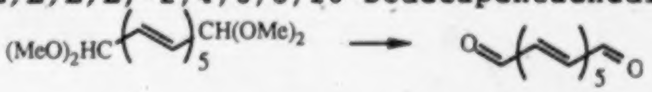
Dibromide **31** (61.4 g, 0.29 mol) and triethyl phosphite were stirred magnetically and heated at 100°C (oil bath). The ethyl bromide that distilled over was condensed and collected; its amount was used to monitor the progress of the reaction. When the initially vigorous reaction had subsided the temperature was raised to 140°C and heating was continued for 24 h. Distillation gave **32** as pale yellow oil (45.37 g, 48% yield): bp 176–180°C (0.45 mm) [lit.^{126} 90%, bp 143°C (0.09 mm)]; ^{1}H NMR (CDCl$_3$, 80 MHz) δ 1.32 (t, J = 7.0 Hz, 12 H), 2.45–2.56 (m, 2 H), 2.68–2.81 (m, 2 H), 4.12 (quintet, J = 7.0 Hz, 8 H), 5.49–5.72 (m, 2 H).

1,1,12,12-Tetramethoxy-2,4,6,8,10-dodecapentaene (33).



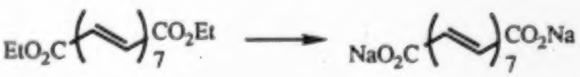
A solution of aldehyde **26** (1.30 g, 10,0 mmol) and phosphonate **32** (1.64 g, 5 mmol) in dry toluene (15 mL) was added dropwise to a magnetically stirred suspension of potassium *tert*-butoxide (1.12 g, 10 mmol) in toluene (20 mL). Stirring was continued until disappearance of aldehyde **26** (TLC, silica, 3:7 ethyl acetate–hexane) was complete, the mixture was poured into cold water (50 mL), and extracted with ether (2 x 50 mL). The combined organic extracts were dried (MgSO$_4$) and evaporated. Flash chromatography of the residue over silica gel (4 x 20 cm) with 3:7 ethyl acetate–hexane gave **33** as a mixture of geometrical isomers (0.37 g, 26%). ^{1}H NMR (CDCl$_3$, 200 MHz) δ 3.30–3.38 (m, 12 H), 4.82–4.95 (m, 2 H), 5.60–6.96 (m, 10 H). The compound was unstable to silica.

(*E,E,E,E,E,E*)-2,4,6,8,10-Dodecapentaenedial (34).



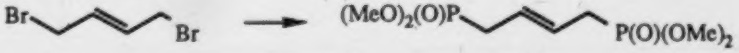
A solution of aldehyde **26** (10.41 g, 80 mmol) and diphosphonate **36** (8.98 g, 40 mmol) in THF (60 mL) was added over 60 min to a stirred and cooled (ice-water bath) solution of potassium *tert*-butoxide (8.98 g, 80 mmol) in THF (150 mL). The cold-bath was removed and stirring was continued for 30 min. Formic acid (10 mL) was then added. Evaporation of the solvents and flash chromatography of the residue over silica gel (6 x 20 cm) with 1:9 ethyl acetate–chloroform gave **34** (2.34 g, 31%) as deep orange, homogeneous (TLC, silica, 1:9 ethyl acetate–chloroform) crystals. An analytical sample was obtained by crystallization from benzene containing a little Norit A: mp 188–189°C [lit.[127] mp 173–174 °C]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.21 (dd, J = 15.0, 8.0 Hz, 2 H), 6.54–6.61 (m, 4 H), 6.71–6.78 (m, 2 H), 7.15 (dd, J = 15.0, 11.0 Hz, 2 H), 9.59 (d, J = 8.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 132.39, 132.72, 136.59, 141.20, 150.60, 193.27; Anal. Calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.34; H, 6.45; exact mass *m/z* calcd for $C_{12}H_{12}O_2$ 188.0837, found 188.0841.

Sodium (*E,E,E,E,E,E,E*) 2,4,6,8,10,12,14-hexadecaheptaenedioate (35).[53]



A solution of sodium hydroxide in methanol (2.5 M, 2 mL) was added to a magnetically stirred solution of diethyl ester **16** (50 mg, 0.154 mmol) in dioxane (50 mL). Stirring was continued for 12 h, the precipitate was collected by centrifugation, washed with methanol (3 mL; centrifugation) and dioxane (3 mL; centrifugation), and dried (oil-pump) to give **35** (45 mg, 0.93 %), which was used without further purification. The material dissolved in water to give a yellow solution.

Tetramethyl (*E*)-but-2-ene-1,4-diylbisphosphonate (36).[128]



Dibromide **31** (45.0 g, 0.210 mol) was added in one portion to trimethylphosphite (52.1 g, 0.420 mol) and the mixture was stirred magnetically and heated at 110°C for 5 h. The cooled reaction mixture was stirred with hexane (100 mL) and then with ether (50 mL) and was left under ether (50 mL) for about 15 h, by which stage the product had solidified. The crude material was purified by distillation under reduced pressure to give **36** (30.0 g, 53 %): bp 180–190°C (0.8 mm); mp 58–60.5 °C [lit.[128] bp 160–162°C (2 mm), mp 58.5–60.5°C]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.64 (ddd, J = 18.0, 4.0, 1.5 Hz, 4 H), 3.76 (d, J = 11.0 Hz, 12 H), 5.59–5.71 (m, 2 H).

Trimethyl phosphonoacetate (38).

$$BrCH_2CO_2Me \longrightarrow (MeO)_2P(O)CH_2CO_2Me$$

Methyl bromoacetate (85.2 g, 0.557 mol) was added dropwise to a magnetically stirred and heated (oil bath, 100°C) portion of triethyl phosphite (69.1 g, 0.557 mol). The rate of addition was controlled so as to maintain moderate boiling and, when the addition was complete, the bath temperature was raised to 150°C and stirring and heating were continued for 5 h. The reaction mixture was distilled under reduced pressure to give trimethyl phosphonoacetate **38** as colorless liquid (91.1 g, 90%): bp 77–79°C (0.1 mm) [lit.[129] 74%, bp 87–90°C (0.2 mm)]; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 3.00 (d, J = 22.0 Hz, 2 H), 3.73 (s, 6 H), 3.94 (s, 3 H).

***tert*-Butyl chloroacetate (39).**[130]

$$ClCH_2COCl \longrightarrow ClCH_2CO_2tBu$$

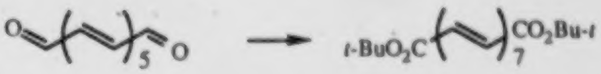
tert-Butyl alcohol (35.4 mL, 27.8 g, 0.38 mol) was added dropwise over 20 min to a magnetically stirred and cooled (ice-bath) mixture of chloroacetyl chloride (30.6 mL, 43.4 g, 0.38 mol) and dimethylaniline (50.0 mL, 47.8 g, 0.39 mol). Stirring was continued overnight and the mixture was poured into water (150 mL) and extracted with ether (2 x 100). The combined organic extracts were washed with saturated aqueous sodium bicarbonate (2 x 30 mL) and water (2 X 30 mL), dried ($MgSO_4$) and evaporated. The residue was distilled to afford **39** (35.3 g, 62%) as a colorless liquid which developed a blue tint after few months' storage : bp 64–66°C (24 mm) [lit.[130] 63%, bp 48–49°C (11 mm)]; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 1.50 (s, 9 H), 3.99 (s, 2 H).

***tert*-Butyl (dimethoxyphosphinyl)acetate (40).**

$$ClCH_2CO_2tBu \longrightarrow (MeO)_2P(O)CH_2CO_2tBu$$

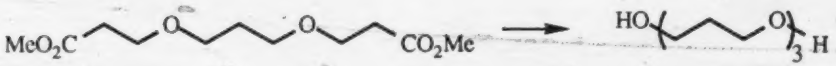
A mixture of **39** (15.04 g, 0.100 mol) and trimethyl phosphite (12.41 g, 0.100 mol) was stirred magnetically and refluxed. The oil bath was kept at 70°C for 10 min, at 100°C for 10 min, at 110°C for 10 min, and at 140°C for 7 h. The residue was distilled under reduced pressure gave pure (^{1}H NMR) **40** as a colorless liquid (14.39 g, 64%): bp 85–87°C (0.06 mm); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 1.22 (s, 9 H), 2.66 (d, *J* = 22.0 Hz, 2 H), 3.56 (d, *J* = 11.0 Hz, 6 H).

Di-*tert*-butyl (*E,E,E,E,E,E,E*) 2,4,6,8,10,12,14-hexadecaheptaenedioate (41).



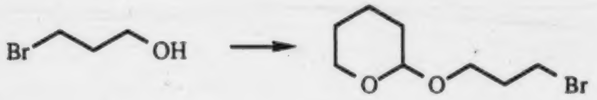
A solution of **40** (0.448 g, 2.00 mmol) in THF (50 mL) was added dropwise over 3 min to a magnetically stirred and cooled (ice-bath) mixture of sodium hydride [(50% w/w suspension in mineral oil, 100 mg, 2.08 mmol) that had been washed with dry hexane (3 x 2 mL)] and THF (50 mL). The cold-bath was removed and stirring was continued until a clear solution formed. A solution of dialdehyde **34** (188 mg, 1.00 mmol) in THF (20 mL) was added dropwise over 20 min and stirring was continued for 2 h. The precipitate was collected by centrifugation, washed with THF (2 x 10 mL; centrifugation), and dried. The crude material was dissolved in dichloromethane and the solution was filtered. The filtrate was evaporated and the solid residue was crystalized from toluene to give pure (TLC, silica, dichloromethane) **41** (105 mg). More product (98 mg) was obtained by evaporation of the mother liquors and flash chromatography of the residue over silica gel (4 x 16 cm) with dichloromethane. The total yield corresponds to 53%: mp 211–213°C; FT-IR ($CHCl_3$ cast) 3014, 1699, 1621, 1592, 1246, 1135, 1015 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 18 H), 5.82 (d, J = 15.0 Hz, 2 H), 6.29–6.51 (m, 8 H), 6.58 (dd, J = 15.0, 10.0 Hz, 2 H), 7.22 (dd, J = 15.0, 11.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 28.15, 80.22, 123.12, 130.88, 133.34, 134.71, 136.43, 139.77, 143.13, 166.40; exact mass *m/z* calcd for $C_{24}H_{32}O_4$ 384.2300, found 328.2299.

4,8-Dioxaundecan-1,11-diol (42).



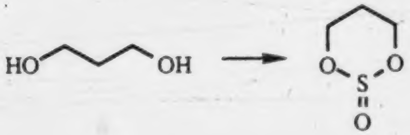
A solution of **87** (505.0 mg, 2.03 mmol) in dry THF (4 mL) was added dropwise over 1 min, under argon, to a magnetically stirred suspension of lithium aluminum hydride (243.0 mg, 6.40 mmol) in THF (15 mL). The mixture was refluxed for 10 min and then stirred at room temperature for 30 min. Ethyl acetate (2 mL) was added slowly, the mixture was stirred for 10 min and concentrated hydrochloric acid was added dropwise with stirring until the mixture became slightly acidic (pH paper). The organic layer was decanted and the residue was suspended in methanol (15 mL) and centrifuged. The combined organic layers were evaporated to give pure (V.P.C) trimeric diol **42** (283 mg, 72%): ^{1}H NMR (CDCl$_3$, 200 MHz) δ 1.68–1.86 (m, 6 H), 3.07 (bs, 2H), 3.47(t, J = 6.1 Hz, 4 H), 3.57 (t, J= 5.9 Hz, 4 H), 3.69 (t, J = 5.8 Hz, 4 H).

Tetrahydro-2-(3-bromopropoxy)-2H-pyran (47).[131]



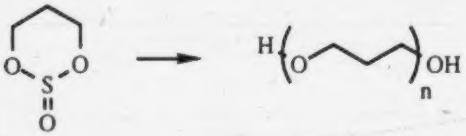
3-Bromo-1-propanol (27.8 g, 0.2 mol) was added dropwise with stirring over 30 min to dihydropyran (20.0 g, 0.238 mol) containing phosphorus trichloride (0.01 mL). Stirring was continued for 4 days and the mixture was distilled to give **47** (24.61 g, 55%) as a pure (^{1}H NMR), colorless liquid: bp 64–5°C (0.25 mm) [lit.[131] 64%, bp 112°C (12 mm)]; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.47–1.90 (m, 6 H), 2.13 (quintet, J = 6.2 Hz, 2 H), 3.47–3.54 (m, 4 H), 3.82–3.91 (m, 2 H), 4.60 (t, J = 3.5 Hz, 1 H).

1,3-Propanediol sulfite (49).[98]



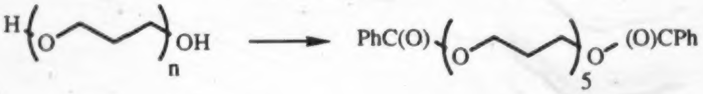
Sulfonyl chloride (119.0 g, 1.00 mol) in carbon tetrachloride (150 ml) was added dropwise over 2.5 h to a magnetically stirred and cooled (ice-water bath) solution of 1,3-propanediol **43** (76.0 g, 1.00 mol) in carbon tetrachloride (80 ml). The rate of addition was controlled so that the temperature remained below 25°C. The cold-bath was replaced with a water-bath and the reaction mixture was warmed rapidly to 70°C. The solvent was then evaporated under reduced pressure and the residue was distilled to give the sulfite **49** (114.5 g , 94%) as a pure (^{1}H NMR), colorless, liquid: bp 55–56°C (5.5 mm Hg) [lit.[98] bp 88°C (39 mm)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.56 (d quintet, J = 14.0, 2.5 Hz, 1 H), 2.32–2.58 (m, 1 H), 3.78 (dm, J = 11.5 Hz, 2 H), 4.85 (dm, J = 11.5 Hz).

Oligomerisation of 1,3-Propanediol sulfite to the mixture of diols (50).



Trimethylenediol (70.3 g, 0.92 mol) was added with magnetic stirring to sodium hydride (55% w/w suspension in mineral oil, 0.44 g, 9 mmol, 1.0% based on diol) followed by trimethylene sulfite **49** (225.7 g, 1.85 mol). The mixture was stirred and refluxed (oil bath set at 200°C). Small samples were withdrawn every hour, distilled in a small Kugelrohr apparatus and analyzed by V.P.C. When all the trimethylene sulfite had been consumed, the mixture was distilled under reduced pressure (0.1 mm, bath temperature up to 200°C) yielding several fractions containing trimethylenediol, its dimer, trimer, and other unidentified components. The total weight of distillate amounted to 85 g. The residue was distilled from a large Kugelrohr apparatus under very low pressure (below 0.001 mm) by raising the Kugelrohr oven temperature from 180°C to 230°C. At this point noticeable decomposition was observed. Several fraction were collected, and each was analyzed by V.P.C. for the content of pentamer. In a typical run 30 g of distillate (from the very high vacuum distillation) contained *ca* 25% of pentamer. This material was separated into its component oligomers after protection of the hydroxyls, the specific method depending on the reaction sequence in question (see below).

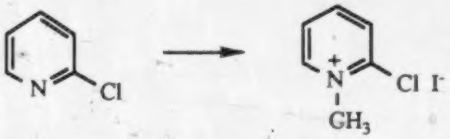
4,8,12,16-Tetraoxanonadecan-1,19-diol dibenzoate (51).



Pyridine (7.90 g, 0.100 mol) and then benzoyl chloride (14.06 g, 0.100 mol) were added to a magnetically stirred solution of diols **50** (16.0 g) in chloroform (100 mL). After 16 h the mixture was washed with aqueous hydrochloric acid (1 M, 2 x 30 mL), saturated aqueous sodium bicarbonate (1 x 30 mL) and water (1 x 30 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the oily residue over silica gel (4 x 25 cm) with chloroform—ethyl acetate mixtures [100:0 (300 mL); 95:5 (200 mL); 10:90 (200 mL); 85:15 (200 mL); 80:20 (100 mL); 70:30 (100 mL)] served to achieve a rough separation of the components and final separation was carried out by HPLC

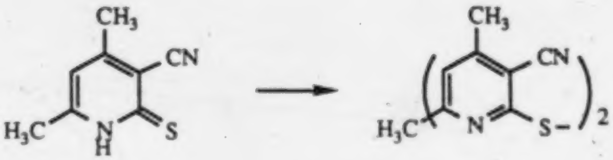
[Waters Prep500A, 100 g silica stainless steel column, 7:3 ethyl acetate–dichloromethane] to give pure (^{1}H NMR) **51** as a pale yellow oil 3.15 g: FT-IR ($CHCl_3$ cast) 2949, 2873, 2869, 2801, 1716, 1601, 1584, 1423, 1315, 1178, 1027, 937, 718, 688, 675 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.73–1.93 (m, 6 H), 2.04 (quintet, J = 6.0 Hz, 4 H), 3.42–3.62 (m, 16 H), 4.42 (t, J = 6.0 Hz, 4 H), 7.38–7.62 (m, 6 H), 8.01–8.10 (m, 4 H); Anal. Calcd for $C_{29}H_{40}O_8$: C, 67.42; H, 7.80. Found: C, 67.03; H, 7.73; MS(CI) *m/z* 534 (M^+ + 18).

2-Chloro-N-methylpyridinium iodide (52).[132]



Methyl iodide (14.2 g, 0.1 mol) was added dropwise to a magnetically stirred and refluxing solution of 2-chloropyridine (11.4 g, 0.1 mol) in acetone. The mixture was refluxed for 24 h and the yellow crystalline salt (5.6 g, 22%) was filtered off and used without further purification: mp 216–217°C (lit.[132] 22%, mp 204–206°C).

2,2'-dithiobis(4,6-dimethyl)-3-pyridinecarbonitrile (53).[133]



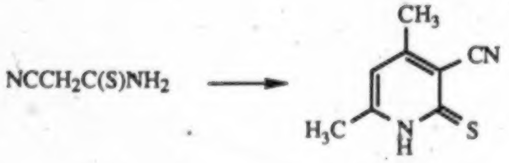
A solution of iodine (3.86 g, 15.2 mmol) in ethanol (95%, 50 mL) was added dropwise to a magnetically stirred solution of **56** (5.0 g, 30.4 mmol) in warm (45 °C) saturated aqueous potassium bicarbonate, and the stirring was continued for 2 h. The yellow precipitate was filtered off and crystalized from 1:1 benzene–butanol to give **53** (2.0 g, 40%): mp 166.5–167.5 °C, [lit.[133] mp 173 °C]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.43–2.46 (m, 12 H), 6.90 (s, 2 H).

2-Cyanoethanothioamide (55).[134]

$$NCCH_2CN \longrightarrow NCCH_2C(S)NH_2$$

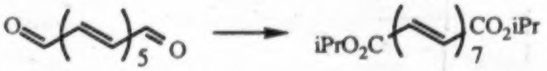
Hydrogen sulfide was passed through a magnetically stirred solution of malonitrile (66.0 g, 1.00 mol) in ethanol (200 mL) containing triethylamine (0.10 mL). A precipitate was formed and, when the slurry became thick, the solid was filtered off and the filtrate was treated again with hydrogen sulfide and the filtrate obtained this time was subjected once more to the reaction conditions. This procedure gave the product as creamy crystals (75.1 g, 75 %): mp (from ethanol) 117–119 [(lit.[134] 80%, mp 118–120 °C)].

1,2-Dihydro-4,6-dimethyl-2-thioxo-3-pyridinecarbonitrile (56).[135]



Triethylamine (0.5 mL) was added to a magnetically stirred solution of 2,4-pentadione (6.0 g, 60 mmol) and **55** (6.0 g, 60 mmol) in ethanol (95%, 100 mL). After the initial exotherm had subsided the mixture was refluxed for 15 min, stirring was continued for 15 min, and the mixture was cooled (ice-bath) for 5 h. The yellow precipitate was filtered off and used without further purification (9.2 g. 93%): mp 256–258°C (dec.) [lit.^{135} mp 250–264 °C (dec.)].

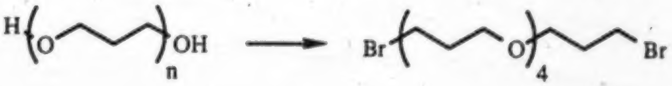
(*E,E,E,E,E,E,E*)-Diisopropyl 2,4,6,8,10,12,14-hexadekaheptaenedioate (57).



Isopropyl iodide (1 mL) was added to a magnetically stirred suspension of sodium salt **35** (45 mg, 0.142 mmol) in HMPA (7 mL) and stirring was continued for 48 h during which time a yellow color developed. The reaction mixture was poured into water (50 mL) and extracted with chloroform (3 x 10 mL). The combined organic extracts were evaporated and flash chromatography of the residue over silica gel (2 x 20 cm) with 46:46:7 hexane–chloroform–ethyl acetate gave pure (TLC, ^{1}H NMR) **57** (5 mg, 10%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (d, J = 6.5 Hz, 12 H), 5.08 (heptet, J = 6.5 Hz, 2 H), 5.87 (d, J =

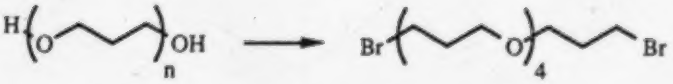
15.0 Hz, 2 H), 6.29–6.54 (m, 8 H), 6.61 (dd, J = 14.5, 10.0 Hz, 2 H), 7.31 (dd, J = 15.0, 12.0 Hz, 2 H).

1,19 Dibromo-4,8,12,16-tetraoxaundecane by bromination of the mixture of diols (58).



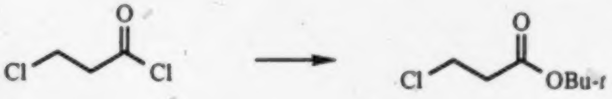
A mixture of diols **50** (250 mg) was added to a magnetically stirred suspension of a triphenylphosphite–bromine complex [obtained *in situ* by addition of a solution of bromine in dichloromethane (23.2%w/w, 1.38 g, 2.0 mmol) to a solution of triphenylphosphate (630 mg, 2.03 mmol) in ether (30 mL)] followed by triethylamine (0.30 mL, 2.15 mmol), and stirring was continued for 3 h. Methanol (3 mL) was added, the mixture was evaporated and the residue was dissolved in ether (20 mL). The solution was washed with water (2 x 5 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1.5 x 15 cm) with dichloromethane–ethyl acetate mixtures [10:90 (75 mL); 75:15 (25 mL); 50:50 (25 mL)] afforded pentamer dibromide **58** as colorless oil (22 mg): ^1H NMR ($CDCl_3$, 300 MHz) δ 1.83 (quintet, J = 6.5 Hz, 6 H), 2.09 (quintet, J = 6.5 Hz, 4 H), 3.44–3.57 (m, 20 H); MS(CI) *m/z* 452 (M^+ + 18).

1,19 Dibromo-4,8,12,16-tetraoxaundecane from the mixture of mesylates (58).



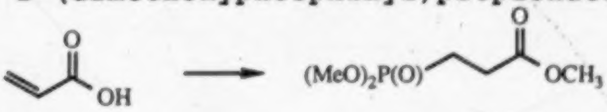
Methanesulfonyl chloride (4.58 g, 40 mmol) was added dropwise to a magnetically stirred and cooled (dry-ice acetone bath) solution of diols **50** (1.00 g) and pyridine (3.18 g, 40 mmol) in dry dichloromethane (40 mL). The cold-bath was removed and stirring was continued at room temperature for 2 h. The reaction mixture was poured into dilute aqueous hydrochloric acid (1 N, 50 mL), the organic layer was separated, washed with water (1 x 20 mL), saturated aqueous sodium bicarbonate (1 x 20 mL) and water (1 x 20 mL), dried ($MgSO_4$) and evaporated under reduced pressure (room temperature, 0.1 mm). The resulting oil was dissolved in isopropanol, lithium bromide (0.70 g, 80 mmol) was added, and the mixture was refluxed for 3 h. The isopropanol was evaporated and flash chromatography of the residue over silica gel (2.5 x 15 cm) with dichloromethane-ethyl acetate mixtures [90:10 (100 mL); 80:20 (200 mL); 70:30 (100 mL)] gave, after evaporation of appropriate (TLC) fractions gave pure product **58** as a colorless oil (371 mg) identical with material obtained earlier.

tert-Butyl 2-chloropropionate(61).



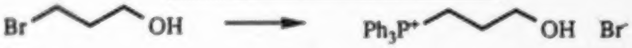
tert-Butyl alcohol (29.6 g, 0.4 mol) was added dropwise with magnetic stirring over 20 min to a cooled (ice-bath) mixture of dimethylaniline (48.5 g, 0.4 mol), 2-chloropropionyl chloride (50.8 g, 0.4 mol) and the rate of addition being controlled so that the temperature did not exceed 20 °C. Stirring was continued for 48 h and the mixture was poured into water (200 mL). The organic phase was separated and washed with aqueous hydrochloric acid (1 x 30 mL), saturated aqueous sodium bicarbonate (1 x 30 mL) and water (1 x 30 mL), dried and distilled to give **61** as colorless liquid (23.0 g, 35%): bp 60–61°C (11 mm) [lit.[136] bp 83–85 °C (17 mm)]; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 1.46 (s, 9 H), 2.72 (t, J = 7.0 Hz, 2 H), 3.76 (t, J = 7.0 Hz, 2 H).

Methyl 2-(dimethoxyphosphinyl)propionate (62).[102]



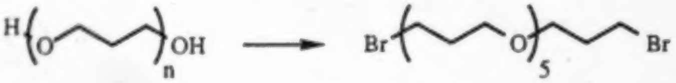
Acrylic acid (21.0 g. 0.29 mol) was added dropwise to a magnetically stirred portion of trimethyl phosphite (36 g, 0.29 mol). After the addition of about three quarters of the acid the temperature rose to 125°C and the rate of addition was controlled to maintain this temperature. Stirring was continued for 2 h at 120 °C (oil bath) and then overnight at room temperature. Distillation afforded pure (^{1}H NMR) **62** as a colorless liquid (36.9 g, 65%): bp 98°C (0.2 mm), [lit.[102] 52%, bp 122°C (3 mm)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.00–2.20 (m, 2 H), 2.53–2.70 (m, 2 H), 3.71 (s, 3 H), 3.77 (d, J = 11.0 Hz, 6 H).

(3-Hydroxypropyl)triphenylphosphonium bromide (63).



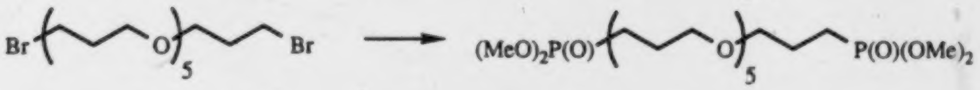
Triphenylphosphine (13.2 g, 50 mmol) was added to a solution of 3-bromopropan-1-ol (7.0 g, 50 mmol) in benzene (35 mL) and the mixture was refluxed for 16 h. The precipitate was filtered off, boiled with acetone (100 mL), filtered off, and crystalized from ethanol (95%) to give **63** (11.74 g, 58%): mp 238–240°C [lit.[103] mp 232.5–233.5 °C]: ^{1}H NMR (CDCl$_3$, 200 MHz) δ 1.82–1.92 (m, 2 H), 3.64–3.88 (m, 4 H), 4.93 (t, J = 7.0 Hz, 1 H), 7.70–7.88 (m, 18 H).

1,23-Dibromo-4,8,12,16,20-pentaoxatricosane from the mixture of mesylates(64).



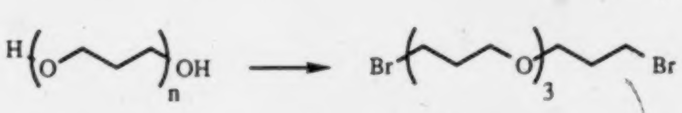
Hexamer dibromide **64** was obtained along with **58** from mixture of diols **50** by mesylation and conversion of the mesylates to bromides with lithium bromide exactly as described above for **58**. After separation by flash chromatography, evaporation of appropriate fractions gave pure **64** as a colorless oil (223 mg): ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.83 (m, 8 H [most likely two overlapping quintets with J = 6.5, 6.5 Hz which are 1.0 Hz apart]), 2.09 (quintet, J = 6.0 Hz, 4 H), 3.45–3.57 (m, 24 H).

Tetramethyl 4,8,12,16,20-pentaoxatricosane-1,23-diylbisphosphonate (65).



Dibromide **64** (505 mg) was added to trimethyl phosphite (3 mL) and the mixture was refluxed for 15 h (oil bath at 160 °C), cooled, and evaporated under reduced pressure (room temperature, 0.1 mm). The oily residue was purified by HPLC [Waters Prep500A, 100 g silica in stainless steel column, 1:19 methanol–dichloromethane] to give pure **65** as a colorless oil (400 mg, 71.5%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.77–1.91 (m, 16 H), 3.42–3.52 (m, 20 H), 3,73 (t, J = 11 Hz, 12 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 20.24, 22.13, 22.60, 22.67, 29.92, 52.06, 52.14, 67.60, 67.68, 67.69, 69.95, 70.17. MS (CI) *m/z* 551 (M+1).

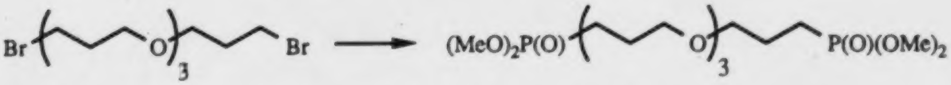
1,15-Dibromo-4,8,12-trioxapentadecane from the mixture of mesylates (66a).



Tetramer dibromide **66a** was obtained along with **58** from a mixture of diols **50** by mesylation and conversion of mesylates to bromides with lithium bromide, exactly as described for

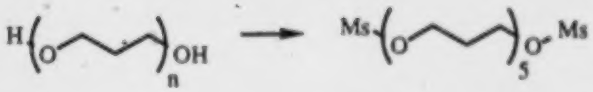
58. After separation by flash chromatography, evaporation of appropriate fractions gave pure **66a** as a colorless oil (301 mg): ^1H NMR ($CDCl_3$, 300 MHz) δ 1.83 (quintet, J = 6.0 Hz, 4 H), 2.09 (quintet, J = 6.0 Hz, 4 H), 3.46–3.57 (m, 16 H); MS(CI) *m/z* 394 (M^+ + 18).

Tetramethyl 4,8,12-trioxapentadecane-1,15-diylbisphosphonate (66).



Dibromide **66a** (0.763 mg, 2.03 mmol) was added to trimethyl phosphite (5 mL) and the mixture was refluxed for 30 h (oil bath at 150 °C). The progress of the reaction was monitored by TLC (silica, 1:19 methanol—dichloromethane) and, when no change was noticed over 10 h, the mixture was cooled and evaporated under reduced pressure (room temperature, 0.1 mm). Flash chromatography of the oily residue over silica gel (2.5 x 20 cm) with 1:19 methanol—dichloromethane gave pure **66** as a colorless oil (549 mg, 63%): ^1H NMR ($CDCl_3$, 300 MHz) δ 1.76–1.92 (m, 12 H), 3.42–3.52 (m, 12 H), 3.74 (d, J = 11.0 Hz, 12 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.41, 22.30, 22.74, 22.81, 30.03, 52.21, 52.30, 67.76, 70.11, 70.34. MS (CI) *m/z* 435 (M+1).

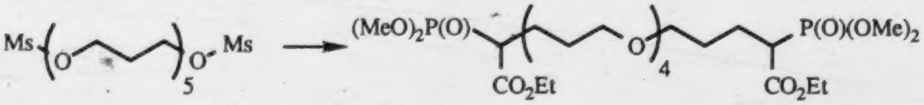
4,8,12,16-Tetraoxaundecan-1,19-diol dimethanesulfonate (68).



Pyridine (11.2 mL, 10.95 g, 0.138 mol) was added to a stirred and cooled (-78°C) solution of the above diol mixture (10.0 g) in dichloromethane (100 mL) and methanesulfonyl chloride (10.8 mL, 15.85 g, 0.138 mol) was added dropwise. Stirring was continued at -78°C for 20 min. The cold-bath was removed and the mixture was placed in a refrigerator for 24 h. Volatile components were evaporated (0.1 mm) at room temperature and the residual thick slurry was dissolved in dichloromethane (10 mL) and chromatographed over silica gel (10 x 15 cm) with dichloromethane—ethyl acetate mixtures [100:0 (200 mL); 90:10 (200 mL); 20:80 (200 mL); 30:70 (400 mL); 40:60 (400 mL); 50:50 (600 mL); 40:60 (400 mL) 30:70 (200 mL); 20:80 (200 mL); 10:90 (200 mL); 0:100 (1000 mL)]. This process achieved a rough separation of the components, and final separation was carried out by HPLC [Waters Prep500A, 1 silica cartridge, 7:3 ethyl acetate—dichloromethane, *ca* 1.0 g samples, each injected in the eluant mixture (2 mL)] to give pure **68** (typical yield *ca* 2.0 g from 10 g of diol mixture). The purity of the product was checked by analytical HPLC (silica, ethyl acetate): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.83 (q, J = 6.5 Hz, 6 H), 2.01 (q, J = 6.5 Hz, 4 H), 3.01 (s, 6 H), 3.44—3.57 (m, 16 H), 4.33 (t, J = 6.5 Hz, 4 H). Anal. Calcd for $C_{17}H_{36}O_{10}S_2$: C, 43.95; H, 7.81; O,34.44; S, 13.80. Found: C, 44.53; H, 7.90; O,33.87;

S, 12.65.; Molecular weight (by vapor pressure osmometry from benzene) Calcd for $C_{17}H_{36}O_{10}S_2$: 464.59 Found: 467.2; MS(CI) *m/z* 386 (M^+ + 18 - CH_3SO_3H).

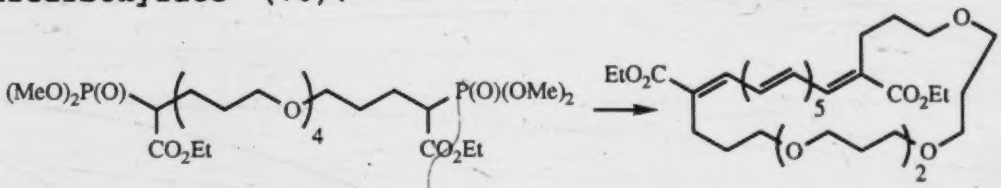
Dimethyl 2,21-bis(dimethoxyphosphinyl)-6,10,14,18-tetraoxatricosanedioate (69).



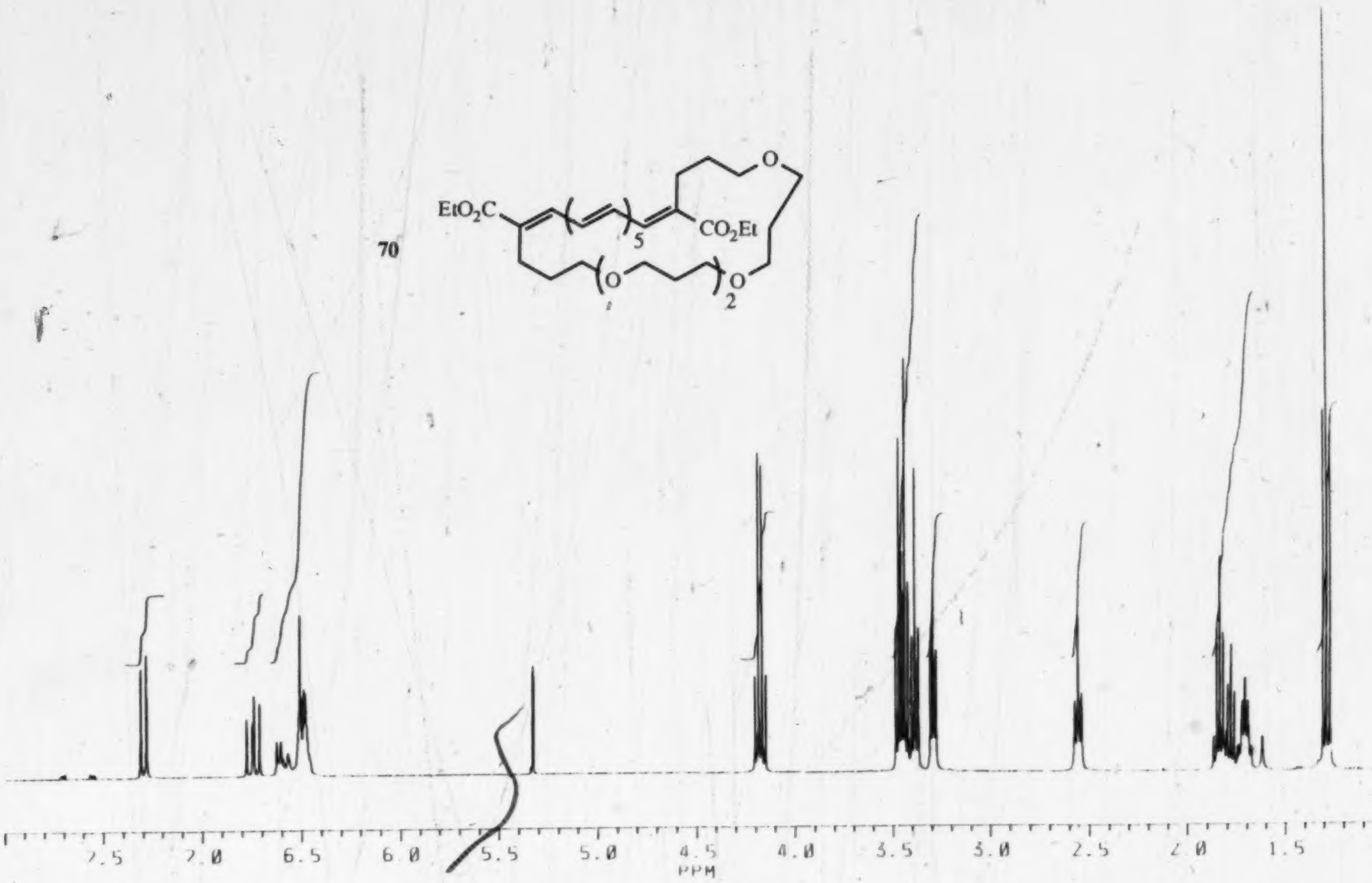
A solution of triethyl phosphonoacetate (790 mg, 3.524 mmol) in THF (15 mL) was added dropwise to a magnetically stirred and cooled (ice-water bath) mixture of sodium hydride [(50% w/w suspension in mineral oil, 300 mg, 6.25 mmol) that had been washed with dry THF (3 x 2 mL)] and THF (5 mL). The reaction was carried out in a pear-shaped flask and the rate of addition was controlled so that the temperature did not exceed 25°C. The cold-bath was removed and stirring was continued for 1 h. The resulting solid was allowed to settle and the clear supernatant was withdrawn into a syringe and added under argon to a stirred solution of dimesylate **68** (405 mg, 0.871 mmol) in THF (5 mL). Stirring was then continued at reflux for 3 days. Methanolic HCl (5% w/w) was added dropwise to bring the pH to 7, and the resulting precipitate was removed by centrifugation. The supernatant was evaporated and flash chromatography of the oily residue over silica gel (4 x 20 cm) with 3:7 acetone—ethyl acetate gave **69** (223 mg, 31%) as colorless, homogeneous (TLC, silica, 3:7

acetone—ethyl acetate) oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.12–1.25 (m, 18 H), 1.40–1.60 (m, 4 H), 1.65–1.98 (m, 10 H), 2.86 (ddd, J = 23.0, 10.0, 5.0 Hz, 2 H), 3.25–3.43 (m, 16 H), 3.97–4.15 (m, 12 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 13.94, 16.13, 16.18, 23.76, 23.80, 28.08, 28.23, 29.90. 44.69, 46.00, 61.10, 62.36, 62.45, 62.52, 67.66, 69.85, 168.92.

Diethyl (*E,E,E,E,E,E,E*) 18,22,26,30-tetraoxa-1,3,5,7,11,13-cyclotritriacontaheptaene-1,14-dicarboxylate (70).



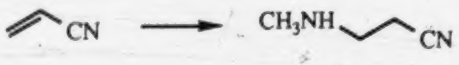
Lithium bis(trimethylsilyl)amide (1.00 M solution in THF, 0.18 mL, 0.18 mmol) was added dropwise to a magnetically stirred and cooled (-78°C) solution of diphosphonate **69** (65.7 mg, 0.091 mmol) in dry THF (5 mL) (argon atmosphere). Stirring was continued for 5 min and the cold-bath was removed. When the mixture had attained room temperature (*ca* 20 min), dry THF was added (to a total volume of 10 mL) and the resulting solution was withdrawn into a syringe. A solution of dialdehyde **34** (17.1 mg, 0.091 mmol) in dry THF (10 mL) was withdrawn into a second syringe (argon atmosphere). Both reagents were injected simultaneously under argon into a magnetically stirred portion (35 mL) of dry THF contained in a flask wrapped with aluminum foil to



70

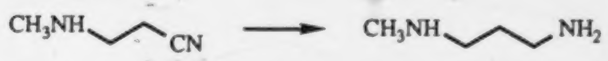
protect the final product from light. The addition took 10 h and was done by syringe pump. Stirring was continued for an additional 4 h. The solvent was evaporated and flash chromatography of the residue over silica gel (2.5 x 15 cm) with mixtures of ethyl acetate and dichloromethane [20:80 (50 mL); 30:70 (100 mL); 40:60 (200 mL)] and mixtures of acetone and dichloromethane [30:70 (50 mL); 100:0 (150 mL)] gave **70** (13.9 mg, 25.0%) as a dark orange solid: mp 91-94°C (without cristallization); ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 1.29 (t, J = 5.0 Hz, 6 H), 1.71 (quintet, J = 5.5 Hz, 4 H), 1.74–1.87 (m, 6 H), 2.55 (t, J = 6.8 Hz, 4H), 3.29 (t, J = 5.5 Hz, 4 H), 3.38 (t, J = 6.6 Hz, 4 H), 3.41–3.48 (m, 8 H), 4.19 (q, J = 5.0 Hz, 4 H), 6.44–6.63 (m, 8 H), 6.74 (dd, J = 14.5, 11.5 Hz, 2 H), 7.29 (d, J = 11.5 Hz, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 14.58, 23.96, 29.63, 30.82, 30.91, 60.81, 68.42, 68.53, 68.72, 69.30, 128.83, 132.13, 132.90, 133.57, 134.83, 138.85, 139.19, 168.18; MS (CI) *m/z* 618 (M + NH_4^+) ; and **71** (14.1 mg, 25.8%) as a dark orange solid: ^{1}H NMR (CD_2Cl_2, 200 MHz) δ 1.24–1.28 (m, 6 H), 1.62–2.00 (m, 10 H), 2.48 (t, J = 6.8 Hz, 2 H), 2.61 (t, J = 6.8 Hz, 2 H), 3.18–3.57 (m, 16 H), 4.14–4.32 (m, 4 H), 6.34–6.85 (m, 10 H), 7.34 (m, 2H).

2-(N-methylamino)propionitrile (74).[105]



Acrylonitrile (106.0 g, 2.00 mol) was added dropwise to a magnetically stirred and cooled (ice-bath) portion of aqueous methylamine (40%w/w, 232 g, 3 mol). The rate of addition was controlled so that the temperature did not exceed 30°C. Stirring was continued overnight, the mixture was refluxed for 0.5 h, and was then cooled. Sufficient anhydrous potassium carbonate was added with stirring to obtain a saturated solution. The top layer was separated and the aqueous layer was extracted with ether (1 x 100 mL). The combined organic layers were dried overnight ($MgSO_4$), the solvent was evaporated and the residue was distilled to give pure **74** as colorless liquid (105.0 g, 62%): bp 86–87°C (25 mm), [lit.[105] 65%, bp 37°C (4 mm)]; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.30 (bs, 1 H), 2.43 (s, 3 H), 2.51 (t, J = 7.0 Hz, 2 H), 2.86 (t, J = 7.0 Hz, 2 H).

N-methyl-1,3-propanediamine (75).[106]



Aminonitrile **74** (21.0 g, 0.25 mol) was added to a suspension of Raney-Nickel catalyst (W-2, prepared according to literature procedure [137]) (*ca.* 3 g) in absolute ethanol contained in a glass-lined steel bomb, and the mixture was saturated with ammonia. The bomb was closed and filled with hydrogen (120 atmospheres) and hydrogenation was carried out at 110°C (120 atmospheres) for 2 h. The catalyst was filtered off and the mixture was distilled through an efficient Vigreaux column to give pure (^1H NMR) **75** as colorless oil (11.0 g, 50%): bp 138–139°C [lit.[106] bp 138–

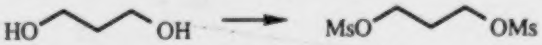
141°C]; ^{1}H NMR (CDCl$_3$, 200 MHz) δ 1.16 (s, 3 H), 1.44 (quintet, J = 7.0 Hz, 2 H), 2.24 (s, 3 H), 2.44 (t, J = 7.0 Hz, 2 H), 2.58 (t, J = 7.0 Hz, 2 H).

tert-Butylphosphorimidic trichloride (76).[138]

$$t\text{-BuNH}_2 \longrightarrow t\text{-BuN=PCl}_3$$

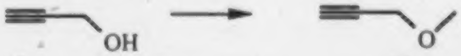
tert-Butylamine (44.0 g, 0.60 mol) was added in small portions to a suspension of phosphorous pentachloride (104.0 g, 0.50 mol) in carbon tetrachloride (150 mL) at such a rate that gentle boiling was maintained. The mixture was refluxed for 100 h (protection from moisture with a drying tube packed with Drierite). During the reaction the solid disappeared and only a small amount was left at the end. Distillation afforded **76** as colorless, very hygroscopic, liquid (36.9 g, 35%). During some runs, small amounts of an unidentified by-product codistilled with the main fraction and was removed by cooling the distillate and filtering off the precipitate that formed: bp 154–156°C [lit.[138] 30%, bp 153–154°C]; ^{1}H NMR (CDCl$_3$, 200 MHz) δ 1.68 (d, J = 5.0 Hz).

Trimethylene diol dimesylate (79)



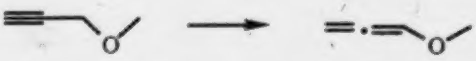
Methanesulfonyl chloride (63.0 g, 0.55 mol) was added dropwise over 0.5 h to a magnetically stirred and cooled (dry-ice acetone bath) solution of trimethylene diol (19.0 g, 0.25 mol) and pyridine (43.5 g, 0.55 mol) in dichloromethane (90 mL). The temperature was maintained at -15°C by intermittent removal of the cold-bath. When the addition was complete the mixture was placed in a refrigerator for 24 h. The solvent was then evaporated and the oily residue extracted with ether (100 mL) and dissolved in dichloromethane (250 mL). The solution was washed with aqueous hydrochloric acid (1 N, 2 x 50 mL), saturated aqueous potassium bicarbonate (1 x 50 mL), dried ($MgSO_4$) and evaporated. The semi-solid crude material was dissolved in ethyl acetate (100 mL) and cooled in dry-ice. The crystalline product was quickly filtered off and washed with cold (dry-ice) ethyl acetate (20 mL) to give pure (^1H NMR) **79** (34.0 g, 60%): mp 39-41°C; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.14 (quintet, $J = 5.9$ Hz, 2 H), 3.00 (s, 6 H), 4.31 (t, $J = 5.9$ Hz, 4 H); Anal. Calcd for $C_5H_{12}O_6S_2$: C, 25.86; H, 5.21; S, 27.61. Found: C, 25.94; H, 5.07; S, 27.33.

Propargylmethyl ether (81).[109]



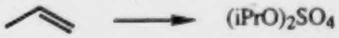
Aqueous sodium hydroxide (50% w/w, 110 g, 1.25 mol) was added dropwise to a magnetically stirred and cooled (ice-bath) mixture of propargyl alcohol (56.0 g, 1.00 mol) and water (44 mL). The rate of addition was controlled to keep the temperature below 35°C. Dimethyl sulfate (75.0 g, 0.60 mol) was then added dropwise and the rate of addition was now controlled to keep the temperature below 55°C. The cold-bath was replaced with an oil bath and stirring was continued under reflux at 55°C. The reflux condenser was replaced with a Liebig condenser and the mixture was distilled (until fumes started to form in the distillation flask) to give pure **81** as a colorless liquid (39.5 g, 56 %): bp 56°C , lit.[110] bp 60-62°C]; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.46 (t, J = 6.0 Hz, 1 H), 3.39 (s, 3 H), 4.10 (d, J = 6.0 Hz, 2 H).

Allenylmethyl ether (82).[110]



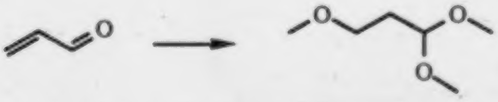
Propargyl methyl ether (39.0 g, 0.556 mol) was added under argon to potassium *tert*-butoxide (6.2 g, 0.05 mol) and the mixture was refluxed with magnetic stirring for 3 h. Volatile components were then distilled off under reduced pressure (water-pump) at room temperature and collected with the use of a dry-ice cold trap. The crude material was distilled to give pure **82** (32.4 g, 83%): bp 47–48°C , lit.[110] 82%, bp 51–52°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.41 (s, 3 H), 3.21 5.47 (d, J = 5.8 Hz, 2H), 6.77 (t, J = 5.8 Hz, 1 H).

Isopropyl sulfate (84a).[111]



Propylene was passed through a magnetically stirred and cooled (dry-ice bath) portion of sulfuric acid (49.0 g, 0.50 mol). The temperature was maintained at -5°C by occasionally lowering the cold-bath, and the gain of weight was checked periodically. When 33.0 g of propylene had been absorbed, the mixture was poured into ice-cold water (200 mL) and quickly extracted with ether (2 x 100 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Distillation of the residue gave pure (^1H NMR) **84a** as a colorless liquid (41.0 g, 58%): bp 72–73°C (0.6 mm), [lit.[111] 64%, bp 50°C (0.2 mm), lit.[139] bp 98°C (2 mm)]; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.46 (d, J = 6.5 Hz, 12 H), 4.89 (heptet, J = 6.5 Hz, 2 H).

1,1,3-trimethoxypropane (84).[112]

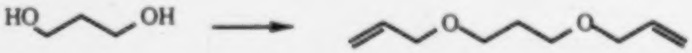


Acrolein (50.0 g, 0.89 mol) was added to a mixture of methanol (200 mL) and benzene (300 mL) containing concentrated hydrochloric acid (1 mL), and the mixture was refluxed for 12 h in a flask equipped with a pressure equalizing addition funnel containing molecular sieves (5Å, 300 g), the funnel being inserted between the distillation flask and the reflux condenser. The solvents were evaporated by distillation at atmospheric pressure, and then distillation under reduced pressure afforded pure **84** as colorless liquid (110 g, 92%): bp 82–83°C (105 mm) [lit.[111] bp 94–95°C(142 mm)]; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.76 (dt, J = 5.8, 6.1 Hz, 2 H), 3.21 (s, 3 H), 3.22(s, 6 H), 3.32 (t, J = 6.1 Hz, 2 H), 4.40 (t, J = 5.8 Hz, 1 H).

Preparation of palladium catalyst.[113]

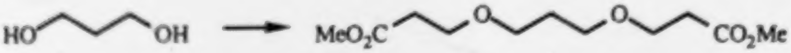
Distilled water (35 mL) was added to a mixture of palladium dichloride (443 mg, 2.50 mmol), cadmium chloride dihydrate (1.14 g, 5.2 mmol) and concentrated hydrochloric acid (5 mL). More distilled water was added (total volume 75 mL) and the mixture was warmed (oil bath, 60°C) and stirred magnetically for 30 min. The resulting solution was subjected to electrolysis using a platinum wire as anode and a copper wire as cathode. A current of 0.1 A was employed at 2.5 V, and occasionally the alloy of palladium and cadmium which deposited on the cathode was washed off with distilled water and collected. After 3 h of electrolysis 340 mg of catalyst had been collected.

Bis-1,3(allyloxo)propane (86).



A solution of 1,3-propanediol (10.0 g, 0.132 mol) in dry THF (25 mL) was added dropwise over 15 min to a magnetically stirred mixture of sodium hydride [(60% w/w suspension in mineral oil, 12.7 g, 0.317 mol) that had been washed with dry hexane (2 x 20 mL)] and THF (100 mL). The mixture was refluxed for 0.5 h, cooled to room temperature, and then allyl bromide (35.1 g, 0.290 mol) in THF (25 mL) was added dropwise over 30 min. The mixture was refluxed overnight, cooled and filtered, and the precipitate was washed with ether (2 x 50 mL). The filtrates were combined, the solvents were evaporated, and the crude product was distilled to give pure (^{1}H NMR) **86** as colorless liquid (18.3 g, 89 %) whose ^{1}H NMR agreed well with the literature data[140]: bp 103–104°C (50 mm); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.85 (quintet, J = 6.0 Hz, 2 H), 3.50 (t, J = 6.0 Hz, 4 H), 3.95 (dt, J = 5.5, 1.5 Hz, 4 H), 5.09–5.31 (m, 4 H), 5.78–5.99 (m, 2 H).

Dimethyl 4,8-dioxaundecanedioate (87).

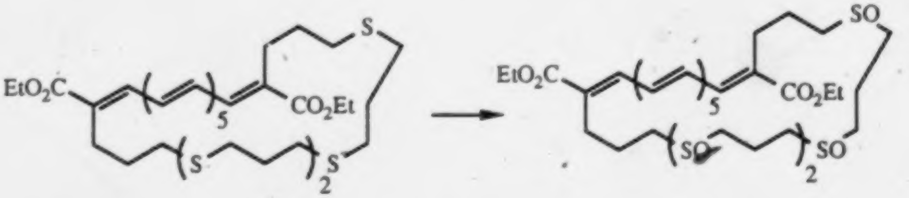


Trimethylene diol (4.00 g, 52.6 mmol), mercury acetate (38.0 g, 119 mmol) and freshly distilled methyl acrylate (30.0 g, 34.8 mmol) were stirred magnetically until almost all of the solid material had dissolved (6 days). Methyl acrylate was evaporated and the oily residue was dissolved in methanol (100 mL). Sodium borohydride (5.0 g, 132 mmol) was added in portions to a magnetically stirred solution and the mixture was cooled with a dry-ice bath so that the temperature was maintained at 0°C. Mercury was removed by centrifugation and the supernatant was evaporated. The resulting thick residue was dissolved in water (100 mL), neutralized with solid potassium bicarbonate (5.0 g) and extracted with ether (1 x 50 mL, 2 x 25 mL). The combined ethereal extracts were the washed with water (20 x 5 mL), dried ($MgSO_4$) and evaporated. Vacuum distillation afforded pure (^{1}H NMR) **87** (4.83 g, 37%). [All aqueous phases were combined and extracted with dichloromethane (10 x 10 mL). The organic layer was dried

($MgSO_4$) and evaporated to give a mixture of starting diol, monoadduct, **87** and a product of addition of acetic acid to methyl acrylate (4.10 g)]. Compound **87** had: bp 121°C (0.2 mm); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.68 (q, J = 6.4 Hz, 2 H), 2.45 (t, J = 6.4 Hz, 4 H), 3.38 (t, J = 6.4 Hz, 4 H), 3.55–3.59 (m, 10 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 29.54, 34.66, 51.36, 65.88, 67.54, 171.82.

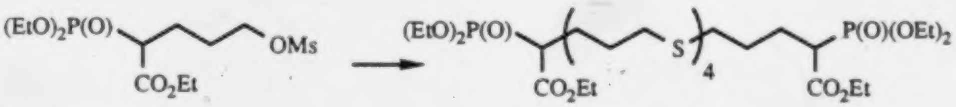
Preparation of sodium metaperiodate on alumina.[120] Acidic alumina (Brockmann No. 1, 33.4 g) was added in one portion to a magnetically stirred solution of sodium periodate (21.4 g, 0.1 mol) in water (60 mL) at 60°C, and the mixture was stirred at this temperature for 20 min. The water was evaporated under reduced pressure (1 mm, 60°C) and the reagent was finally dried in an oven (24 h, 120°C).

***(E,E,E,E,E,E,E)*-Diethyl 18,22,26,30-tetrathiaoxo-1,3,5,7,11,13-cyclotritriacontaheptaene-1,14-dicarboxylate (90).**



Sodium metaperiodate on alumina (150 mg) was added to a stirred solution of **102** (14.0 mg, 0.0211 mmol) in a mixture of methanol (2.5 mL) and THF (2.5 mL). The progress of the reaction was monitored closely by TLC (silica, methanol). As soon as formation of sulfones was detected (observation of a new spot with R_f higher than that of **90**), the solid reagent was removed by centrifugation and the supernatant was diluted with dichloromethane (20 mL), and washed with water (3 x 5 mL). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 15 cm) with methanol gave **90** (6.9 mg, 45%) as an apparently homogeneous (TLC, silica, methanol), red-brown solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30 (t, J = 7.1 Hz, 6 H), 1.96–2.26 (m, 10 H), 2.45–2.55 (m, 2 H), 2.60–2.80 (m, 18 H), 4.19 (q, J = 7.1 Hz, 4 H), 6.44–6.72 (m, 10 H), 7.33–7.37 (m, 2 H); MS (FAB) *m/z* 729 (M+1).

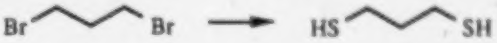
Dimethyl 2,21-bis(dimethoxyphosphinyl)-6,10,14,18-tetrathiatricosanedioate (91).



Ethanol (95%, 20 mL),followed by a solution of sodium ethoxide in ethanol [(1.00 M, 1.22 mL) prepared by dissolving sodium metal (575 mg, 0.025 mol) in ethanol (25.00 mL of solution)], was added with magnetic stirring to dimercaptan **98** (156.9 mg, 0.612 mmol). More ethanol (20 mL) was added to dissolve all the mercaptan and the solution was cooled to -78°C. Mesylate **101** (468 mg, 1.298 mmol) in ethanol (5 mL) was added in one portion, the cold-bath was removed, and stirring was continued overnight. Evaporation of the solvent

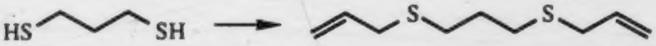
(1 mm, room temperature) and flash chromatography of the residue over silica gel (2.5 x 15 cm) with acetone–dichloromethane mixtures [10:90 (100 mL); 15:75 (200 mL); 20:80 (700 mL)] gave **91** (205 mg, 43.0%) as a pure (^{1}H NMR), colorless oil: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.23 (t, J = 7.0 Hz, 6 H), 1.27 (dt, J = 2.5, 7.0 Hz, 12 H), 1.47–2.27 (m, 14 H), 2.40–2.49 (m, 4 H), 2.49–2.58 (m, 12 H), 2.87 (ddd, J = 23.0, 11.0, 4.0 Hz, 2 H), 4.04–4.22 (m, 12 H); ^{13}C NMR (CDCl$_3$, 100.6 MHz) δ 14.03, 16.26, 26.17, 26.21, 28.20, 28.34, 29.36, 30.96, 31.49, 44.83, 46.14, 61.24, 62.58, 168.84. Anal. Calcd for $C_{31}H_{62}O_{10}P_2S_4$: C, 47.43; H, 7.96; S,16.34. Found: C, 47.28; H, 7.70; S,16.55.

1,3-Propanedithiol (92).



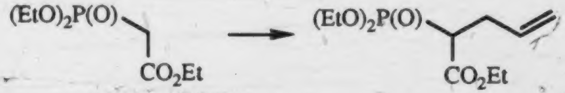
Thiourea (38 g, 0.50 mol) was added with magnetic stirring to 1,3-dibromopropane (38.4 g, 0.19 mol), followed by water (25 mL). The stirred mixture was refluxed for 3.5 h. A solution of sodium hydroxide (30.0 g, 0.75 mol) in water (300 mL) was added. The mixture was again refluxed for 5 h and left overnight at room temperature. A solution of concentrated. sulfuric acid (13 g, 0.13 mol) in water (50 ml) was added and the product was extracted with ether (2 x 100 mL). The combined ethereal layers were dried (MgSO$_4$) and evaporated, and the residue was distilled to give **92** (14.4 g, 70%) as a 95% pure (V.P.C.), colorless, liquid: bp 166°C [lit.[141] 169°C]; ^{1}H NMR (CDCl$_3$, 200 MHz) δ 1.35 (t, J = 8.0 Hz, 2 H), 1.91 (quintet, J = 7.0 Hz, 2 H), 2.67 (dt, J = 8.0, 7.0 Hz, 4 H).

Bis-1,3(allylthio)propane (93).



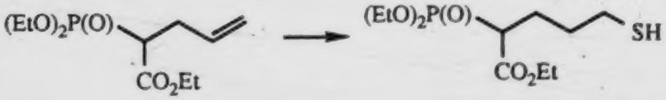
Sodium (3.12 g. 0.136 mol) was dissolved in ethanol (95%, 50 mL) and 1,3-propanedithiol **92** (7.00 g. 0.0647 mol) was added followed by a solution of allyl bromide (18.8 g, 0.155 mol) in ethanol (95%, 40 ml). The mixture was refluxed for 2 h and was then evaporated. The residue was dissolved in ether (100 mL), the precipitate was filtered off, the solvent was evaporated, and the crude material was distilled under reduced pressure to give **93** (8.75 g, 72%) as a pure (^{1}H NMR), colorless, oil: bp 109–110°C (0.45 mm Hg); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.82 (quintet, J = 7.0 Hz, 2 H), 2.55 (t, J = 7.0 Hz, 4 H), 3.11–3.14 (m, 4 H), 5.07–5.13 (m, 4H), 5.73–5.84 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 28.37, 29.07, 34.34, 117.09, 134.61; Anal. Calcd for $C_8H_{16}S_2$: C, 57.39; H, 8.56; S, 34.05. Found: C, 57.46; H, 8.60; S, 34.39.

Ethyl 2-(diethoxyphosphinyl)pent-4-enoate (94).



Triethyl phosphonoacetate (11.2 g, 50.0 mmol) was added dropwise over 20 min to a magnetically stirred and cooled (ice-bath) mixture of sodium hydride [(60% w/w suspension in mineral oil, 2.0 g, 50.0 mmol) that had been washed with dry THF (2 x 7 mL)] and THF (40 mL). The cold-bath was removed and stirring was continued until a clear solution formed. The reagent was transferred to an addition funnel and added dropwise over 1 h to a magnetically stirred, refluxing solution of allyl bromide (12.1 g, 0.10 mol) in THF (30 mL). Refluxing was continued for 3 h. The resulting precipitate was filtered off, and the filtrate was evaporated. Distillation of the residue using a spinning band apparatus [Perkin-Elmer 151 Annular Still] gave **94** (3.6 g, 27%). The material was found to be 90% pure (V.P.C.) and had: bp 90°C (0.3 mm); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.17 (t, J = 7.0 Hz, 3 H), 1.24 (dt, J = 3.5, 7.0 Hz, 6 H), 2.43–2.53 (m, 1 H), 2.53–2.67 (m, 1 H), 2.92 (ddd, J = 23.0, 12.0, 4.0 Hz, 1 H), 3.98–4.14 (m, 6 H), 4.91–5.04 (m, 2 H), 5.60–5.72 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 13.95, 16.15, 16.18, 30.80, 30.84, 44.61, 45.92, 61.15, 62.46, 62.52, 62.58, 62.64, 116.89, 134.35, 134.51, 168.36.

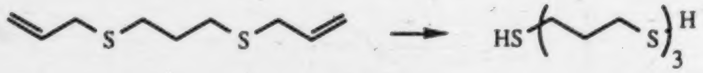
Ethyl 2-(diethoxyphosphinyl)-5-mercaptopentanoate (97).



A solution allyl derivative **94** (0.516 g, 1.96 mmol) in dry hexane (3 mL), contained in a quartz flask marked at the 3-

and 6-mL levels and fitted with a dry-ice condenser, was cooled to -78°C. Hydrogen sulfide was passed in. The gas condensed on the cold surface and dropped into the solution. Passage of hydrogen sulfide was stopped when the solution volume had increased by 3 mL. The dry-ice condenser was left in place and the flask was irradiated with U.V. light (Rayonet photochemical reactor lamp, catalogue No. RPR 3000 Å) for 1.5 hours. Occasionally, ice which built up on the surface of the flask was washed off with acetone. The dry-ice condenser was removed and, when the reaction mixture had attained room temperature, the volatile components were evaporated by a current of nitrogen. The oily residue was purified by flash chromatography over silica gel (4.5 x 20 cm) with ethyl acetate—dichloromethane mixtures [2:8 (200 mL); 3:7 (200 mL); 4:6 (100 mL); 1:1 (400 mL)] to give **97** as pure (^{1}H NMR) colorless oil (420 mg, 72%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.14—1.29 (m, 10 H), 1.47—1.63 (m, 2 H), 1.79—2.02 (m, 2H), 2.42 (q, J = 7.5 Hz, 2 H), 2.81 (ddd, J = 23.0, 10.0, 5.0 Hz, 1 H); exact mass, *m/z* calcd for $C_{11}H_{23}OPS$ 298.1003, found 298.1006.

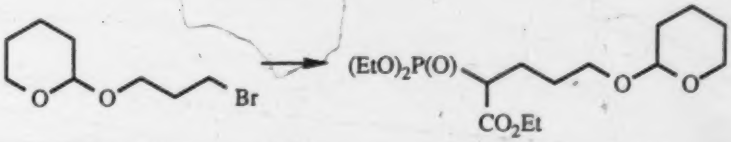
Bis-1,3(3-mercaptopropanethio)propane (98).



A solution of the diallyl bis-sulfide **93** (1.52 g, 8.072 mmol) in dry THF (10 mL), contained in a quartz flask, marked at the 10- and 25-mL levels, and fitted with a dry-ice condenser, was cooled to -78°C. Hydrogen sulfide was passed in. The gas condensed on the cold surface and dropped into the solution. Passage of hydrogen sulfide was stopped when the solution volume had increased by 15 mL. The dry-ice condenser was left in place and the flask was irradiated with U.V. light (Rayonet photochemical reactor lamp, catalogue No. RPR 3000 Å) for 3 h. Occasionally, the ice which built up on the surface of the flask was washed off with acetone. The dry-ice condenser was removed and, when the reaction mixture had attained room temperature (*ca* 45 min), the volatile components were evaporated by a current of nitrogen. The oily residue was dissolved in ether (50 mL) and extracted with 1.5 N sodium hydroxide (3 x 15 mL). The organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in dry THF (10 mL) and addition of hydrogen sulfide and aqueous workup were repeated as before. The combined basic aqueous extracts from both runs were acidified with concentrated hydrochloric acid and extracted with ether (3 x 15 mL). The combined ethereal extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the oily residue over silica gel (2.5 x 15 cm) with 1:2 petroleum ether–dichloromethane gave **98** (1.06 g, 28%) as a homogeneous (TLC, silica, 2:1 petroleum ether–dichloromethane), colorless oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 (t, J = 8.0 Hz, 2 H), 1.74–1.84 (m, 6 H), 2.51–2.60 (m, 12 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 23.30, 29.22, 30.30, 30.78, 33.23. Anal. Calcd for $C_9H_{20}S_4$:

C, 42.14; H,7.86; S, 50.00. Found: C, 42.42; H,7.81; S, 50.04.

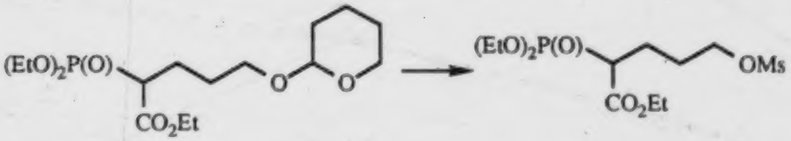
Ethyl 2-(diethoxyphosphinyl)-5-(2H-tetrahydropyranyloxy)pentanoate (99).



A solution of triethyl phosphonoacetate (9.129 g, 40.7 mmol) in THF (20 mL) was added dropwise to a magnetically stirred and cooled (ice-water bath) mixture of sodium hydride [(60% suspension in mineral oil, 1.71 g, 42.8 mmol) that had been washed with dry THF (3 x 5 mL)] in THF(60 mL). The rate of addition was controlled so that the temperature did not exceed 25°C. The cold-bath was removed and stirring was continued until a clear solution had formed. Bromide **47** (9.41 g, 40.7 mmol) in THF (10 mL) was added dropwise over 15 min, the mixture was refluxed for 24 h, and stirring at room temperature was continued for three days. The solvent was evaporated and the oily residue was extracted with ether (3 x 15 mL). The combined ethereal extracts were centrifuged to remove residual solid and evaporated to yield the crude product as a pale yellow oil (15.6 g, 104%), which was used without further purification. An analytical sample was obtained by chromatography over alumina (2.5 x 20 cm) with 3:7 ethyl acetate—dichloromethane: ^{1}H NMR ($CDCl_3$, 400 MHz) δ

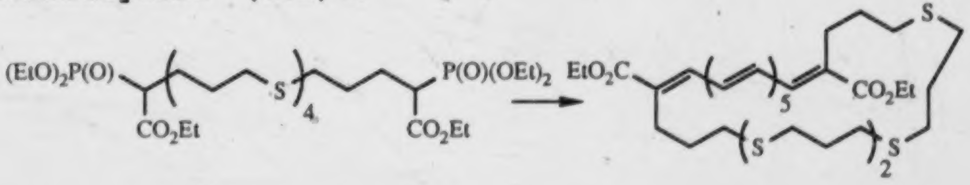
1.22 (t, J = 7.0 Hz, 3 H), 1.27 (dt, J = 3.0, 8.0 Hz, 6 H), 1.43–2.04 (m, 10 H), 2.94 (ddd, J = 23.0, 11.0, 4.0 Hz, 1 H), 3.32 (dq, J = 10.0, 7.0 Hz, 1 H), 3.40–3.47 (m, 1 H), 3.68 (dq, J = 10.0, 6.5 Hz, 1H), 3.74–3.81 (m, 1 H), 4.04–4.21 (m, 6 H), 4.50 (t, J = 3.5 Hz, 1 H). Anal. Calcd for $C_{16}H_{31}O_7P$: C, 52.45; H, 8.53. Found: C, 52.32; H, 8.60.

Ethyl 2-(diethoxyphosphinyl)-5-mathanesulfonylpentanoate (101).



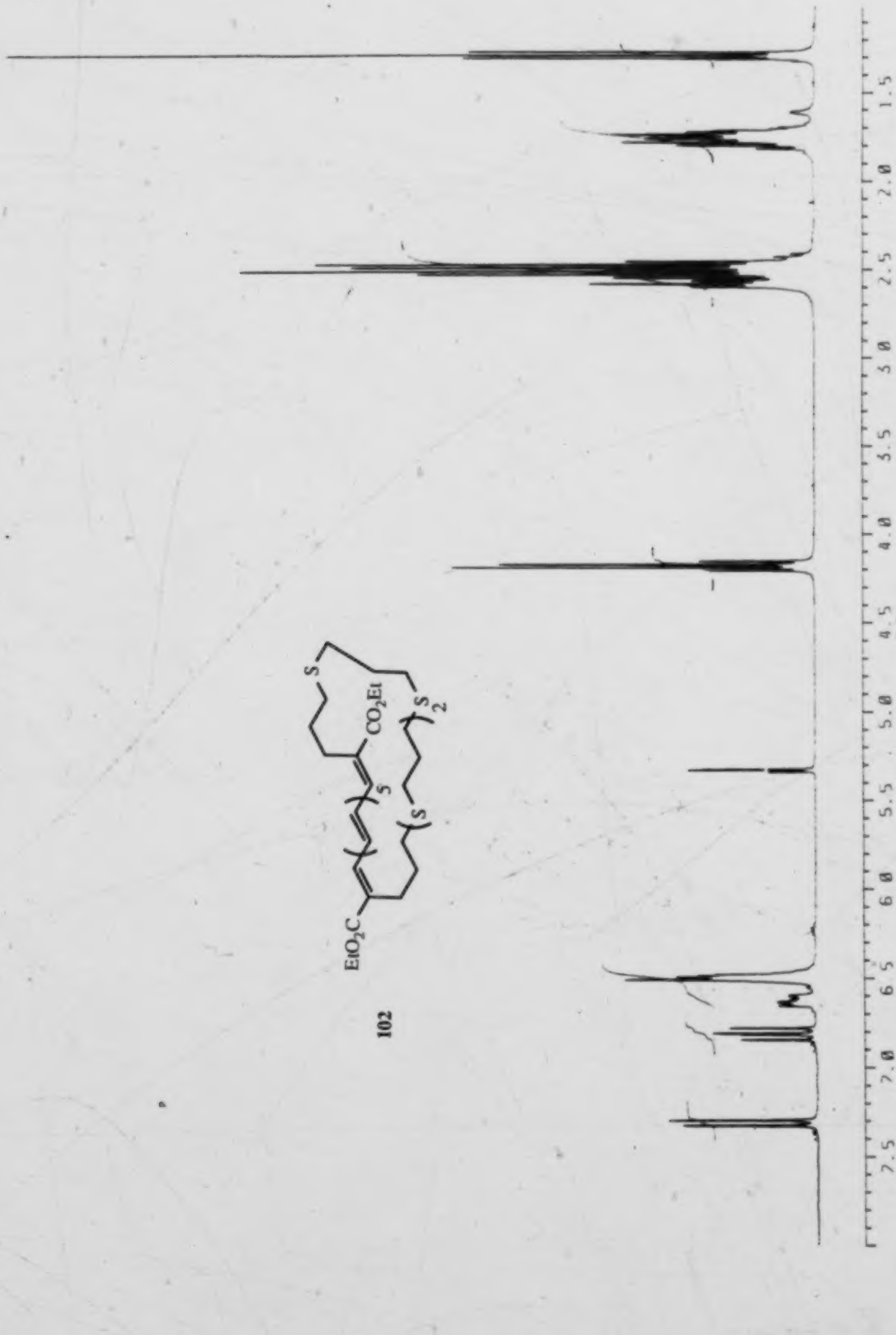
Compound **99** (3.663 g, 10.0 mmol) was added to a suspension of acidic resin (Amberlite IR-120, 100 mg) in ethanol (95%, 30 mL) and the mixture was refluxed for 3 h. The resin was removed by filtration and the solvent was evaporated under reduced pressure (0.5 mm, room temperature). The oily residue was dissolved in dichloromethane (25 mL) and cooled to -78°C. Pyridine (2.34 g, 30 mmol) was added dropwise at a fast rate, with stirring, followed by methanesulfonyl chloride (3.43 g, 30 mmol). The cold-bath was removed and the mixture was kept overnight in a refrigerator. The volatile components were evaporated under reduced pressure (0.5 mm, room temperature) and flash chromatography of the residue over silica gel (5 x 12 cm) with mixtures of dichloromethane and ethyl acetate [80:20 (200 mL); 60:40 (400 mL); 0:100 (700 mL)], gave fractions containing the desired product along with some other materials. Appropriate fractions (TLC) were combined and evaporated and the product was isolated by HPLC [Waters Prep500A, 1 silica cartridge, 7:3 ethyl acetate–dichloromethane, *ca* 1.0 g samples, each injected in the eluant mixture (2 mL)] to give **101** (1.60 g, 44.5%) as a pure (analytical HPLC, silica, acetone), colorless oil: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.20–1.30 (m, 9 H), 1.67–2.08 (m, 4 H), 2.84–2.98 (m, 4 H), 4.02–4.20 (m, 8 H); ^{13}C NMR (CDCl$_3$, 100.6 MHz) δ 13.98, 16.22, 23.18, 27.62, 27.76, 37.37, 44.32, 45.63, 61.46, 62.84, 86.79, 168.53. Anal. Calcd for $C_{12}H_{25}O_8SP$: C, 40.00; H, 7.00; S, 8.90. Found: C, 39.66; H, 6.78; S, 8.82.

(E,E,E,E,E,E,E)-Diethyl 18,22,26,30-tetrathia-1,3,5,7,11,13-cyclotritriacontaheptaene-1,14-dicarboxylate (102).



Lithium bis(trimethylsilyl)amide (1.00 M solution in THF, 0.25 mL, 0.25 mmol) was added dropwise to a magnetically stirred and cooled (-78°C) solution of diphosphonate **91** (97.6 mg, 0.124 mmol) in dry THF (5 mL) (argon atmosphere). Stirring was continued for 5 min and the cold-bath was removed. When the mixture had attained room temperature (*ca*

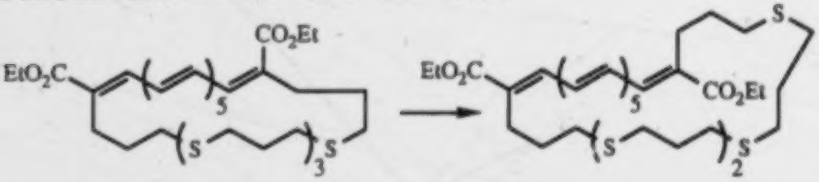
30 min), dry THF was added (to a total volume of 8 mL) and the resulting solution was withdrawn into a syringe. A solution of dialdehyde **34** (23.4 mg, 0.124 mmol) in dry THF (8 mL) was withdrawn into a second syringe (argon atmosphere). Both reagents were injected simultaneously under argon into a magnetically stirred portion (30 mL) of dry THF contained in a flask wrapped with aluminum foil to protect the final product from light. The addition took 12 h and was done by syringe pump. Stirring was continued for an additional 4 h. The solvent was evaporated and flash chromatography of the residue over silica gel (2.5 x 15 cm) with 3:97 ethyl acetate—dichloromethane gave **103** (less than 0.2 mg) as a dark orange solid: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.30(t, J = 7.2 Hz), 4.21 (q, J = 7.2 Hz) [Other signals were not clearly identifiable]; **104** (5.0 mg, 6.0%) as a dark orange solid: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.29 [q, J = 7.0 Hz, 6 H (most likely superimposition of two triplets at 1.28 and 1.31 with J = 7.0, 7.0 Hz)], 1.67–1.87 (m, 10 H), 2.43–2.62 (m, 20 H), 4.15–4.24 [m, 4 H (most likely superimposition of two quartets at 4.19 and 4.24 with J = 7.0, 7.0 Hz)], 6.35–6.62 (m, 8 H) 6.64 (d, J = 12.0 Hz), 6.81 (dd, J = 15.0, 12.0 Hz, 1 H), 7.26–7.36 [m, 2 H (most likely including a doublet at 7.30 with J = 12.0 Hz and a doublet of doublets at 7.32 with J = 12.0, 15.0 Hz)]; and **102** (33.6 mg, 40%) as a dark orange solid: mp 110-115°C (without cristallization), 135-137°C (dec.) (from nitromethane); ^{1}H NMR (CD$_2$Cl$_2$, 400 MHz) δ 1.28 (t, J = 7.0 Hz, 6 H), 1.68–1.82 (m, 10 H), 2.40–2.61 (m, 20



102

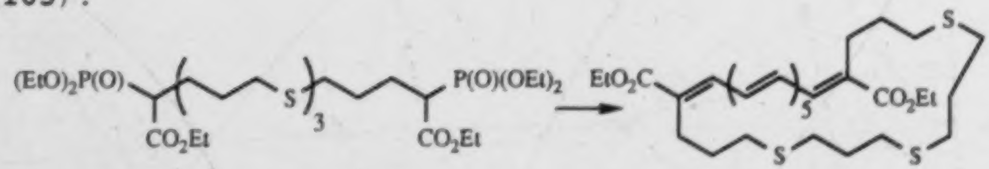
H), 4.17 (q, J = 7.0 Hz, 4 H), 6.42–6.68 (m, 8 H), 6.80 (dd, J = 14.5, 12.0 Hz, 2 H), 7.30 (d, J = 12.0 Hz, 2 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 14.50, 25.70, 28.91, 29.79, 30.12, 31.49, 31.65, 31.70, 31.79, 60.84, 129.09, 131.78, 133.70, 134.39, 135.62, 139.19, 167.93.

Isomerization of 104 to 102.



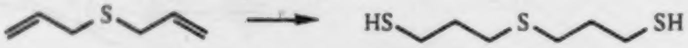
A solution of iodine (0.1% w/v, 50 μL) in dichloromethane was added to a solution of impure fractions of **104** (19 mg) and the mixture was warmed briefly to its boiling point. The progress of the reaction was followed by TLC (silica, 3:97 ethyl acetate–dichloromethane) and, when no further change was observed (10 min), the product was separated by flash chromatography (without prior evaporation of the reaction solvent) over silica with 3:97 ethyl acetate–dichloromethane to give pure (TLC) **102** (12.7 mg, 67%).

***(E,E,E,E,E,E,E)*-Diethyl 18,22,26-trithia-1,3,5,7,11,13-cyclononacosaheptaene-1,14-dicarboxylate (105).**

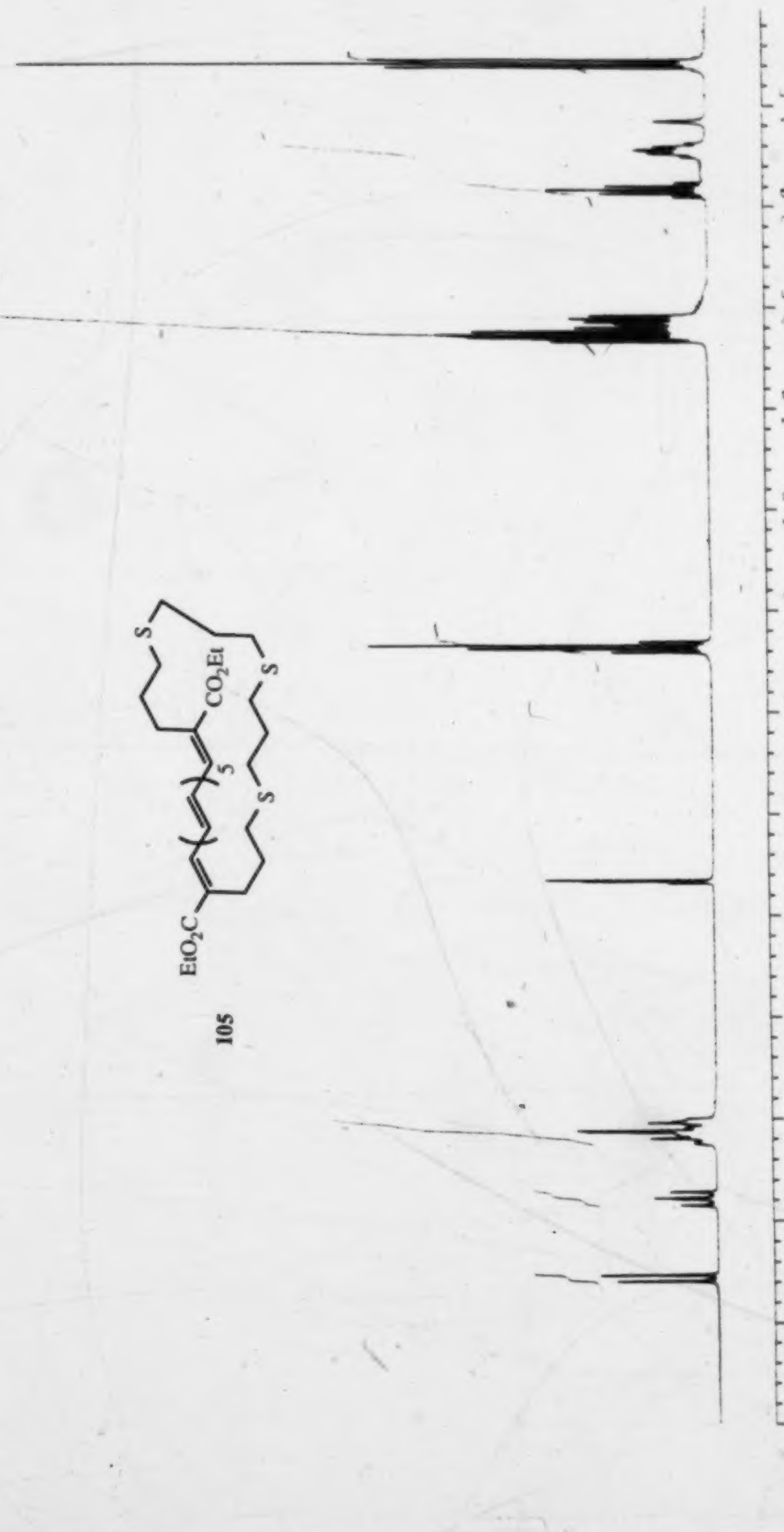


The exact procedure used for cyclization of **91** and **34** was followed using lithium bis(trimethylsilyl)amide (1.00 M solution in THF, 0.21 mL, 21 mmol), diphosphonate **108** (72.8 mg, 0.102 mmol), and dialdehyde **34** (19.3 mg, 0.103 mmol). After workup, evaporation of the solvent and flash chromatography of the residue over silica gel (2.5 x 15 cm) with 2:98 ethyl acetate—dichloromethane gave **105** (23.4 mg, 40% yield) as a dark orange solid: ^1H NMR (CD_2Cl_2, 400 MHz) δ 1.28 (t, *J* = 7.1 Hz, 6 H), 1.72 (b quintet, *J* = 6.8 Hz, 4 H), 1.91 (quintet, *J* = 7.2 Hz, 4 H), 2.36—2.67 (m, 16 H), 4.17 (q, *J* = 7.1 Hz, 4 H), 6.45—6.63 (m, 8 H), 6.88 (dd, *J* = 15.0, 12.0 Hz, 2 H), 7.27(d, *J*= 12.0 Hz, 2 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 14.58, 25.83, 29.40, 30.46, 31,85, 32.70, 60.91, 128.72, 132.39, 132.74, 133.36, 134.33, 138.40, 138.78, 168.07; MS (FAB) *m/z* 590 (M^+).

3,3'-(Thiobis)propanethiol (107).



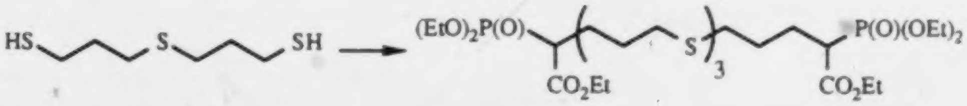
A solution of diallyl sulfide (3.0 g, 26.3 mmol) in dry THF (10 mL) contained in a quartz flask, marked at the 10- and 25-mL levels, and fitted with dry ice condenser, was cooled to -78°C. Hydrogen sulfide was passed in. The gas condensed on the cold surface and dropped into the solution. Passage of hydrogen sulfide was stopped when the solution volume had increased by 15 mL. The dry-ice condenser was left in place and the flask was irradiated with U.V. light (Rayonet photochemical reactor lamp, catalogue. No. RPR 3000 Å) for 3



105

h. Occasionally, the ice which built up on the surface of the flask was washed off with acetone. The dry-ice condenser was removed and, when the reaction mixture had attained room temperature, the volatile components were evaporated by a current of nitrogen. The oily residue was dissolved in ether (50 mL) and extracted with 1.5 N sodium hydroxide (3 x 15 mL). The combined basic aqueous extracts were acidified with concentrated hydrochloric acid and extracted with ether (3 x 15 mL). The combined ethereal extracts were evaporated and flash chromatography of the residue over silica gel (2.5 x 15 cm) with 2:1 petroleum ether—dichloromethane gave **107** as a colorless oil (230 mg, 5%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.31 (t, J = 8.0 Hz, 2 H), 1.83 (quintet, J = 7.0 Hz, 4 H), 2.54–2.66 (m, 8 H). Additionally some monoadduct was separated (600 mg). No attempt was made to separate the nonacidic components of the reaction mixture nor to improve the yield by recovery of starting material or by recycling the monoadduct.

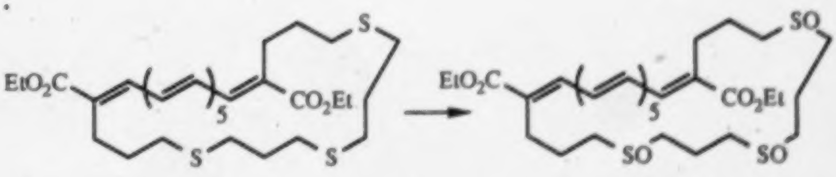
Dimethyl 2,21-bis(dimethoxyphosphinyl)-6,10,14-trithianonadecanedioate (108).



Ethanol (95%, 5 mL),followed by a solution of sodium ethoxide in ethanol [(1.00 M, 0.51 mL, 0.51 mmol) prepared by dissolving sodium metal (575 mg, 0.025 mol) in ethanol (25.00

mL of solution)], was added with magnetic stirring to dimercaptan **107** (46.3 mg, 0.254 mmol). More ethanol (15 mL) was added to dissolve all the mercaptan and the solution was cooled to -78°C. Mesylate **101** (192 mg, 0.533 mmol) in ethanol (3 mL) was added in one portion, the cold-bath was removed, and stirring was continued overnight. Evaporation of the solvent (1 mm, room temperature) and flash chromatography of the residue over silica gel (2.5 x 15 cm) with acetone—dichloromethane mixtures [1:9 (100 mL); 2:8 (200 mL); 3:7 (200 mL); 4:6 (100 mL)] gave **108** (72.8 mg, 40.3%) as a pure (^{1}H NMR), colorless oil: ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 1.25 (t, J = 7.0 Hz, 6 H), 1.29 (dt, J = 2.5, 7.0 Hz, 12 H), 1.50—1.69 (m, 4 H), 1.81 (quintet, J = 7.0 Hz, 4 H), 1.86 (m, 4 H), 2.42—2.52 (m, 4 H), 2.52—2.61 (m, 8 H), 2.90 (ddd, J = 23.0, 11.5, 4.0 Hz, 2 H), 4.06—4.23 (m, 12 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 14.00, 16.22, 26.03, 26.08, 28.07, 28.21, 29.10, 30.70, 31.28, 44.64, 45.95, 61.24, 62.56, 168.88.

(*E,E,E,E,E,E,E*)-Diethyl 18,22,26-trithiaoxo-1,3,5,7,11,13-cyclononacosaheptaene-1,14-dicarboxylate (109).



Sodium metaperiodate on alumina (150 mg) was added to a stirred solution of **105** (3.2 mg, 0.0054 mmol) in a mixture of methanol (1.5 mL) and THF (1.5 mL). The progress of the reaction was monitored closely by TLC (silica, methanol). As soon as formation of sulfones was detected (observation of a new spot with R_f higher than that of **109**), the solid reagent was removed by centrifugation and the supernatant was diluted with dichloromethane (20 mL), and washed with water (3 x 5 mL). Evaporation of the solvent and flash chromatography of the residue over silica gel (1 x 20 cm) with 1:9 methanol–dichloromethane mixture gave **109** (1.0 mg, 29%) as an apparently homogeneous (TLC, silica, methanol), red-brown solid: ^1H NMR ($CD2Cl_2$, 400 MHz) δ 1.29 (t, J = 7.5 Hz, 4 H), 1.88–2.10 (m, 4 H), 2.19–2.37 (m, 4 H), 2.50–2.83 (m, 16 H), 4.19 (q, J = 7.5 Hz, 4 H), 6.47–6.63 (m, 8 H), 6.70–6.78 (m, 2 H), 7.33 (d, J = 12.0 Hz, 2 H); MS (FAB) *m/z* 639 (M+1).

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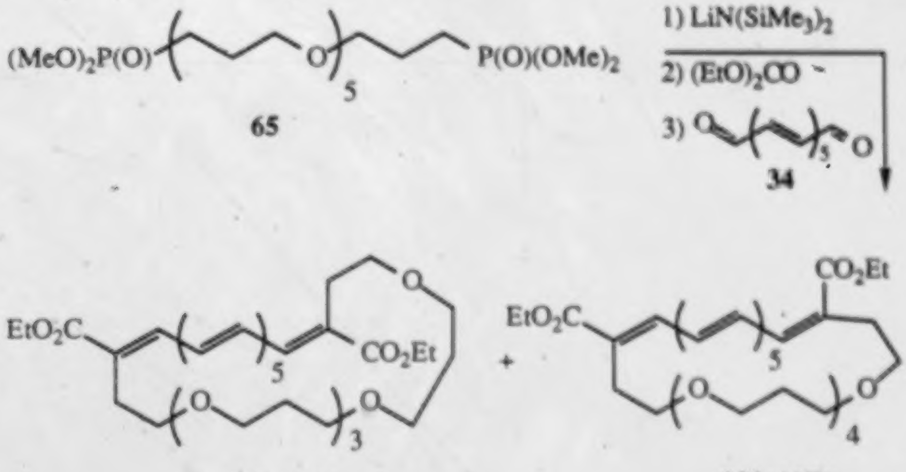
APPENDIX A

As already mentioned in the main part of this thesis, none of the model compounds was active against yeasts. Therefore, we decided to prepare a polyhydroxy model **112** which would resemble the natural material more closely.

This undertaking required some changes from our previous plan. In our synthesis of **70** we had accepted a low yield in the step leading to the required diphosphonate **69**, and had done so because the precursor **68** was readily accessible. However, we felt that the corresponding precursor for the polyhydroxy series might not be readily accessible and so we first sought an improved route to the stabilized diphosphonate that would be used in the final cyclization.

Our previous attempts to improve the yield in the alkylation of triethyl phosphonoacetate with dimesylate **68** were unsuccessful; however, the conversion of dibromides to diphosphonates (for example **64**->**65**) proceeds in quite good yields, even without optimization. Therefore, we focused our attention on possible ways of direct conversion of such diphosphonates to the stabilized analogs. Fortunately, there is methodology available for functionalizing phosphonates at the α-position with carboalkoxy group[1]. The procedure involves reaction of a lithiated phosphonate with diethyl carbonate, and has been used to obtain several substituted phosphonoacetates. It was used also for a direct synthesis of a series of β-substituted acrylates.[1] We decided to explore this possibility with diphosphonate **65**. There are two possible strategies: the synthesis and isolation of a stabilized phosphonate, followed by cyclization in a way analogous to our previous work, or direct cyclization of the diphosponate reagent generated *in situ*. We chose to explore the latter. The reaction (Eq. 28) proceeded as required and we obtained two isomers corresponding to **110** and **111**.



65

34

110 (6%)

111 (4%)

Eq. 28

Although the yield was lower than in the previously used approach, this level of efficiency was obtained without optimization in the present case. Some more polar material, apart from the cyclized products, was observed in the reaction. Possibly this is the monoadduct. We measured the ^{1}H NMR spectra of **110** and **111** and recognized all the characteristic features of the previously obtained macrocycles. Based on the appearance of the unsaturated region, signals corresponding to ethyl substituents, as well as on the character of signals corresponding to allylic protons (one set only), we assigned the isomer **110** an all-trans geometry. The compound **111**, whose ^{1}H NMR spectrum shows two sets of the allylic protons, was assigned the geometry corresponding to **71**, that is, with one terminal double bond cis, and the other one, trans.

Having established a possible alternative for the final cyclization, the preparation of the polyhydroxy unit was begun. First of all, we had to decide where to place the hydroxyl groups. It seemed natural to follow the 1,3 pattern found in the polyene antibiotics and we then had to chose the stereochemical relationship of these hydroxyls. It was not possible to decide whether the relative stereochemistry of the hydroxyl groups of AmB is of importance as AmB is the only polyene antibiotic whose stereochemistry has been established. Visual inspection of CPK models as well as simple modelling with the use of MM2 calculations[a] suggests that the all-syn isomer should have all the hydroxyl groups available for interactions with either water or another molecule of such a macrocycle. (Fig. 8). The next issue to be addressed is the length of the hydrophilic unit and the number of hydroxyl groups. We decided arbitrarily to attach five hydroxyl groups. As far as the length of this unit is

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[a] As previously, we used Chem3D program on a Macintosh II computer.

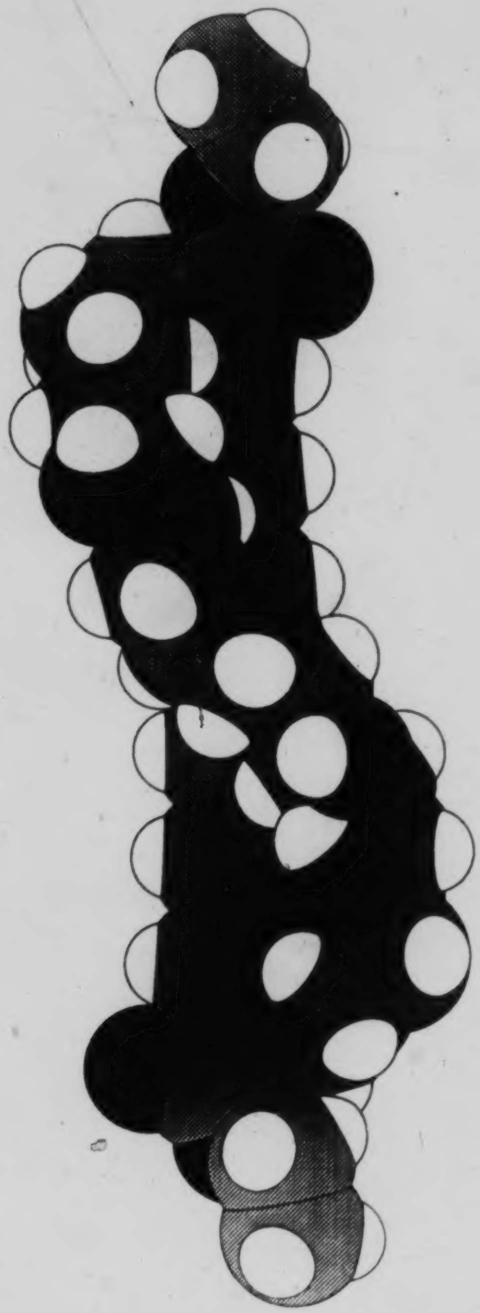
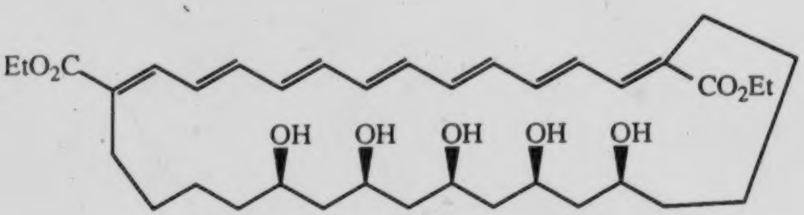


Fig. 8. CPK model of **112**

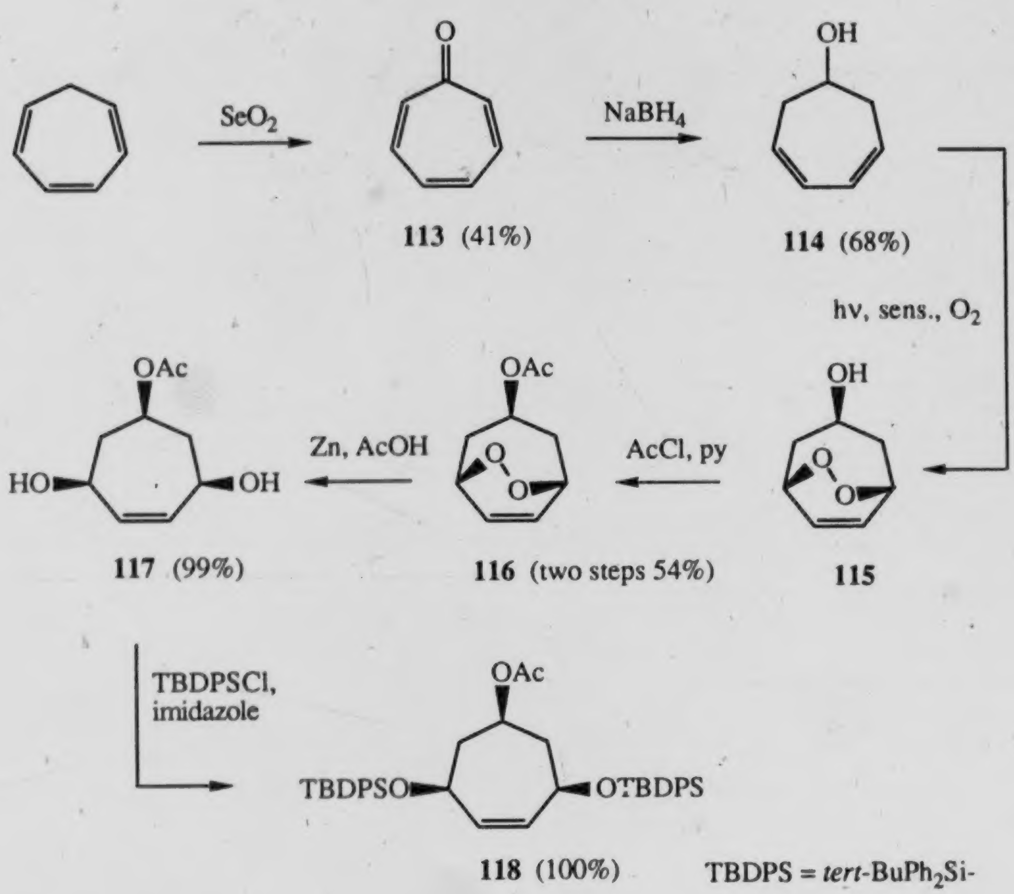
concerned, there are two factors involved. The chain should be sufficiently short to ensure an elongated shape of the molecule (*cf.* **102** and **105**). There is actually a hidden benefit in having the hydrophilic chain short. In our previous synthesis we had noticed that in the case of compound **105**, only one isomer was obtained, whereas in all other cases two or three isomers were formed. Inspection of Dreiding models reveals that, with the shortening of this chain, there is considerably more strain in isomers possessing terminal cis double bonds. Therefore, with a shorter chain, the preferred all-trans isomer would be favored. However, as the hydroxyl groups should be protected during cyclization, the potential that steric hindrance induced by these protecting groups might interfere with the cyclization had to be considered. We decided to settle for a medium size macrocycle with a hydrophilic chain of 17 atoms, and so our target molecule was defined as **112**.



112

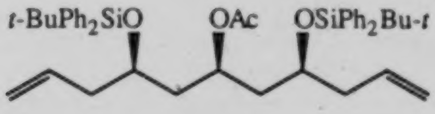
There is a considerable amount of chemical literature devoted to stereospecific synthesis of 1,3-diols.[2-4] Most of the techniques employ an iterative process which includes a stereospecific step or steps, such as the Sharpless epoxidation and opening of the epoxide with an organocopper reagent. However, there is one example[5] of the synthesis of an all-syn triol, in a very efficient way. The synthesis is based on a stereospecific addition of singlet oxygen to cycloheptadienol and we decided to use this method.

Scheme 43


O
SeO_2
$NaBH_4$
OH
113 (41%)
114 (68%)
hv, sens., O_2
OAc
OAc
OH
Zn, AcOH
AcCl, py
HO
OH
O
O
O
O
117 (99%)
116 (two steps 54%)
115
TBDPSCl, imidazole
OAc
TBDPSO
OTBDPS
118 (100%)
TBDPS = *tert*-$BuPh_2Si$-

In fact, there is an example[6] of the synthesis of a 1,3-polyol which starts with the same substrate. The first steps of our plan are shown on Scheme 43.

Oxidation of cycloheptatriene to tropone **113** according to the literature procedure,[7,8] followed by reduction with sodium borohydride[9] afforded dienol **114**. Addition of singlet oxygen gave a mixture of products with the main component being the endoperoxide **115**. This material was not isolated; instead, the mixture was acetylated and the acetate **116** was then purified. We observed somewhat lower yields in the transformations (**114->115->116**) than reported in the literature, and considerably longer times of addition of singlet oxygen (two days, as opposed to 6--8 h). However, we used an ordinary bulb as a source of light and the procedure calls for a halogen lamp. The acetate **116** is a crystalline material and is quite stable at room temperature. We measured its ^{13}C NMR spectrum and it corresponded well with the literature data.[10] Next, we attempted reduction of the peroxide group of **116** according to the literature procedure,[10] (Zn, $ZnCl_2$, MeOH), but we obtained a mixture of products. We tried to reduce the material directly to a triol with the use of $LiAlH_4$ but the yield was rather low and some other products were also formed. DIBAL afforded a mixture of products as well, but use of zinc metal in the presence of acetic acid in dichloromethane[6] gave the diol **117** quantitatively. Protection of the hydroxyl groups of **117** with *tert*-butyldiphenylchlorosilane in the presence of pyridine and dimethylaminopyridine (DMAP) is very slow; however, use of imidazole, as reported by Schreiber[6], yields the disilylated derivative **118** efficiently. Next, we planned to open the cyclic material by ozonolysis. Reductive workup, followed by mesylation, conversion of the resulting dimesylate to a diepoxide, and, reaction with vinylcopper, should give compound **119**.

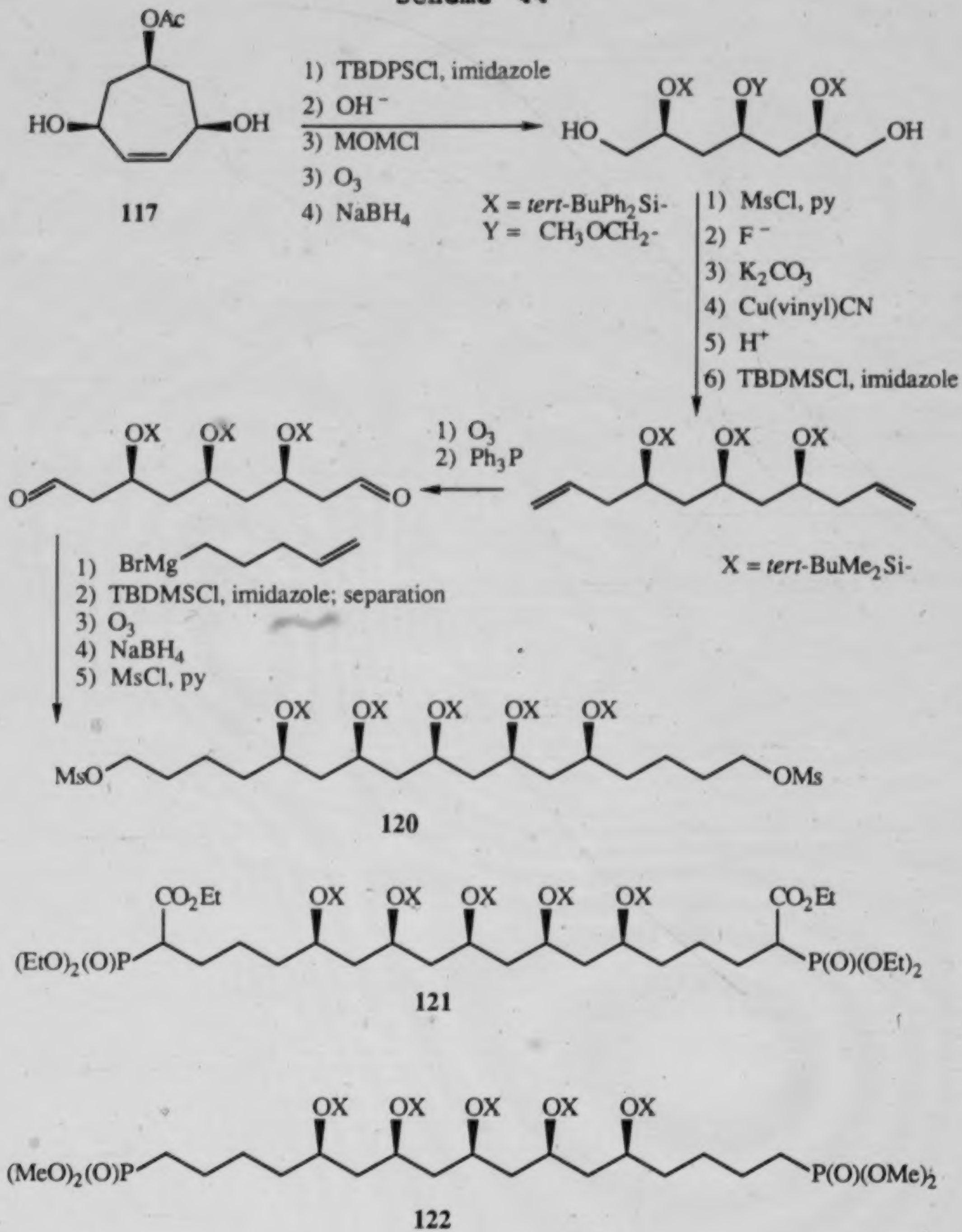


119

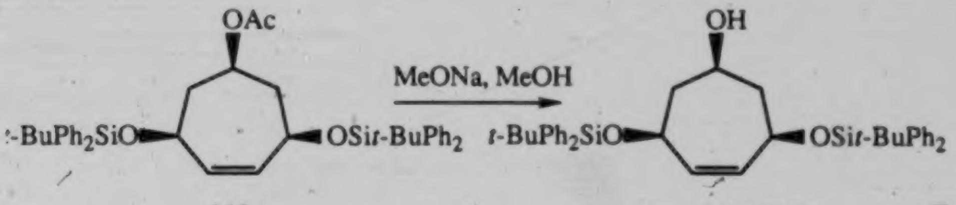
Unfortunately, under the conditions used for the reduction of the ozonide, the acetyl group was partially cleaved and we obtained a mixture of materials.

Our revised plan and further transformations necessary to obtain the desired macrocycle **112** are shown in Scheme 44. We intend to use dimesylate **120** to alkylate triethylphosphonoacetate to obtain the stabilized diphosphonate **121**. This would be cyclized with dialdehyde **34**. Alternatively, **120** could be converted to diphosphonate **122** and a cyclization with the use of diethyl carbonate would then be attempted.

Scheme 44

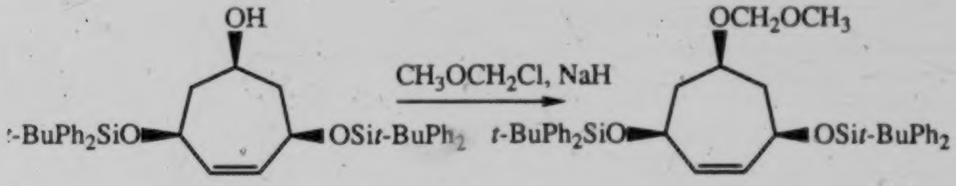

OAc
HO
OH
117
1) TBDPSCl, imidazole
2) OH⁻
3) MOMCl
3) O₃
4) NaBH₄
OX OY OX
HO
OH
X = tert-BuPh₂Si-
Y = CH₃OCH₂-
1) MsCl, py
2) F⁻
3) K₂CO₃
4) Cu(vinyl)CN
5) H⁺
6) TBDMSCl, imidazole
OX OX OX
X = tert-BuMe₂Si-
1) O₃
2) Ph₃P
OX OX OX
O
O
1) BrMg
2) TBDMSCl, imidazole; separation
3) O₃
4) NaBH₄
5) MsCl, py
OX OX OX OX OX
MsO
OMs
120
CO₂Et
OX OX OX OX OX
CO₂Et
(EtO)₂(O)P
P(O)(OEt)₂
121
OX OX OX OX OX
(MeO)₂(O)P
P(O)(OMe)₂
122

Cleavage of acetate **118** with methanolic sodium methoxide (Eq. 29), in THF or ether, proceeded smoothly and we obtained alcohol **123** in good yield.


OAc
MeONa, MeOH
t-BuPh$_2$SiO
OSit-BuPh$_2$
OH
t-BuPh$_2$SiO
OSit-BuPh$_2$

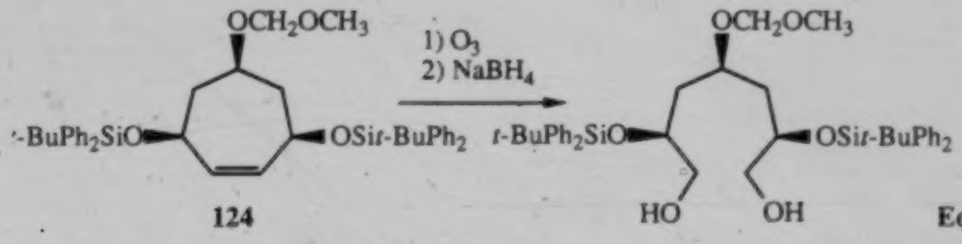
118 **123** (99%) **Eq. 29**

However, protection of the hydroxyl group as methoxymethyl ether was rather difficult. Reaction of alcohol **123** with a large excess of methoxymethyl chloride (Eq. 30) in the presence of sodium hydride in refluxing THF was very slow and the conversion was not complete.


OH
CH$_3$OCH$_2$Cl, NaH
t-BuPh$_2$SiO
OSit-BuPh$_2$
OCH$_2$OCH$_3$
t-BuPh$_2$SiO
OSit-BuPh$_2$

123 **124** (45%) **Eq. 30**

Additionally, under these conditions, partial cleavage of the silyl group was also observed. As a result, the yield of the required material **124** was rather low. Use of imidazole as a base led to formation of a complex mixture and an even lower yield of **124**. Next, we carried out the ozonolysis of **124** followed by reductive workup with sodium borohydride (Eq. 31).

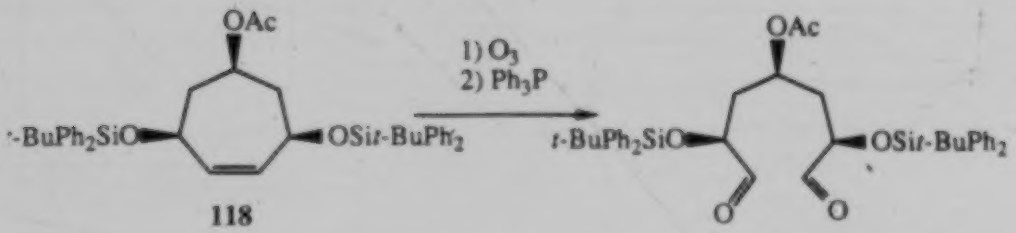


124

125 (30%)

Eq. 31

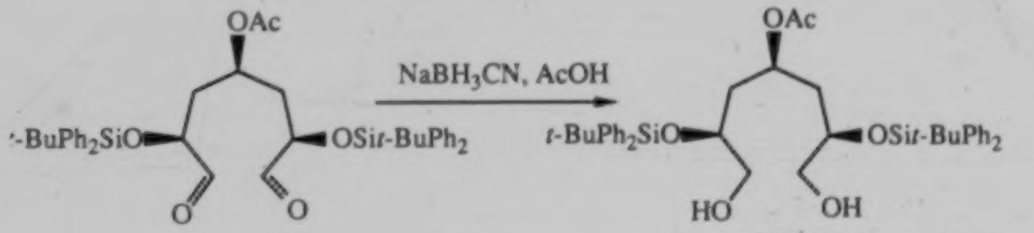
It seems that the ozonide involved is quite stable under these conditions; after 2 h of reduction we did not see any radical change in the composition of the reaction mixture (TLC) and assumed that the reduction was complete. However, the material isolated by flash chromatography decomposed on TLC plates. We resubjected the substance to the reductive conditions and measured the ^{1}H NMR of a sample of this product. The product obtained after 16 h of reduction had a ^{1}H NMR spectrum consistent with the expected diol **125** and different from that of the previously isolated material. However, the yield was low. This low yield for two consecutive steps prompted us to reexamine the transformations of acetate **118** under different conditions. Ozonolysis followed by reduction with triphenylphosphine (Eq. 32), now gave dialdehyde **126** in good yield and the reduction was complete in a few minutes.


1) O_3
2) Ph_3P
OAc
OSit-BuPh2
t-BuPh2SiO

118

126 (90%) **Eq. 32**

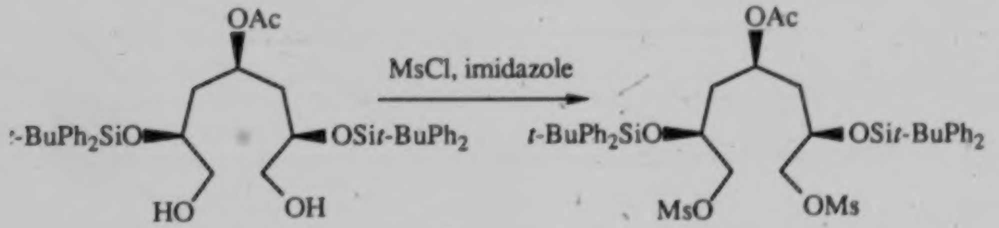
Attempts to reduce the dialdehyde **126** with sodium borohydride led again to partial cleavage of the acetate but reduction under acidic conditions with sodium cyanoborohydride gave the expected diol **127** in good yield.(Eq. 33).


NaBH3CN, AcOH
OAc
OSit-BuPh2
t-BuPh2SiO
HO
OH

126

127 (98%) **Eq. 33**

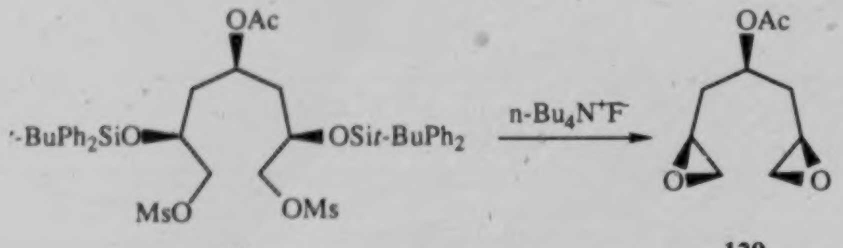
Next, we carried out the mesylation of **127** (Eq. 34).


MsCl, imidazole
OAc
OSit-BuPh2
t-BuPh2SiO
HO
OH
MsO
OMs

127

128 (66%) **Eq. 34**

Under conditions used previously for mesylation of primary alcohols (methylene chloride, mesyl chloride, pyridine, 0°C) the reaction did not proceed at all; at room temperature it was very slow and only after using a large excess of mesyl chloride and imidazole as a base was the expected dimesylate **128** formed at appreciable speed. We hoped that it would be possible to remove the silyl protecting groups and form the epoxide **129** at the same time, by using anhydrous conditions. The reaction with potassium fluoride in the presence of 10% 18-crown-6 was very slow and so we used tetrabutylammonium fluoride instead, except that the THF solution of this reagent was first stirred for 30 min with molecular sieves.



128 **129**

Eq. 35

We indeed obtained the expected epoxide **129** (Eq. 35) as judged by the ^{1}H NMR, but the product was contaminated with some other material inseparable by flash chromatography, and the yield was low. At this point we knew that the source of most of the problems encountered so far, was the use of acetate as a protecting group at the beginning of the synthesis. This choice was, however, compatible with our first plan to carry out homologation of dialdehyde **126** in a Wittig manner and we decided to try this approach now. If this reaction should fail, than a sequence of reactions similar to that shown in Scheme 44 but with the top hydroxyl group protected as benzyl ether (instead of methoxymethyl ether) and the remaining hydroxyl groups protected as less bulky silyl ethers, should be successful.

It seems as well that if the polyhydroxy analog does not exhibit biological activity, there is still one possible modification that could easily be made to the model substance. If the cyclization is carried with the *tert*-butyl analog of the stabilized phosphonate **121**, it may be possible to convert the resulting di-*tert*-butyl ester to the corresponding diacid under fairly mild conditions. This would equip the model with the last characteristic feature of AmB, that is, a polar group.

Experimental.

(E,E,E,E,E,E,E)-Diethyl 17,21,25,25,26-pentaaoxa-1,3,5,7,11,13-cyclopentatriacontaheptaene-1,14-dicarboxylate (110).

Lithium bis(trimethylsilyl)amide (1.00 M solution in THF, 0.50 mL, 0.50 mmol) was added dropwise to a magnetically stirred and cooled (-78°C) solution of diphosphonate **65** (69.3 mg, 0.126 mmol) in dry THF (5 mL) (argon atmosphere). Stirring was continued for 0.5 h and ethyl carbonate (0.031 ml, 29.8 mg, 0.252 mmol) was added rapidly, the mixture was stirred for an additional 0.5 h at -78°C and the cold bath was removed. When the mixture had attained room temperature (*ca* 20 min), dry THF was added (to a total volume of 8 mL) and the resulting solution was withdrawn into a syringe. A solution of dialdehyde **34** (23.7 mg, 0.126 mmol) in dry THF (10 mL) was withdrawn into a second syringe (argon atmosphere). Both reagents were injected simultaneously under argon into a magnetically stirred portion (30 mL) of dry THF contained in a flask wrapped with aluminum foil to protect the final product from light. The addition took 14 h and was done by syringe pump. The mixture was then neutralized by addition of 1N hydrochloric acid and the solvent was evaporated. Flash chromatography of the residue over silica gel (1.5 x 20 cm) with mixtures of ethyl acetate and dichloromethane [30:70 (50 mL); 35:65 (50 mL); 40:60 (100 mL)] gave **110** (5.5 mg, 6.0%) and **111** (3.8 mg, 4 %) as a dark orange solids. Compound **110** had: ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 1.29 (t, J = 7.0 Hz), 1.64--1.81 (m, 8 H), 2.65 (t, J = 5.6 Hz, 4 H), 3.22--3.39 (m, 16 H), 3.39--3.50 (m, 8 H), 4.18 (q, J = 7.0 Hz, 4 H), 6.38--6.65 (m, 8 H), 6.71 (dd, J = 14.5, 11.0 Hz, 2 H), 7.34 (d, J = 11.0 Hz, 2 H); Compound **111** had: ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 1.30 (q, J = 7.3 Hz), 1.64--1.80 (m, 8 H), 2.51 (t, J = 5.2 Hz), 2.65 (t, J = 5.2 Hz), 3.22--3.59 (m, 20 H), 4.14--4.24 (m, 4 H), 6.35--6.68 (m, 9 H), 6.71 (dd, J = 14.8, 11.5 Hz, 1 H), 7.31--7.36 (m, 2 H). A mixture (6 mg) of both isomers was also isolated in this experiment.

Tropone (113).[7,8]

A magnetically stirred mixture of cycloheptatriene (47.0 g, 90%w/w, 0.46 mole), selenium(IV) dioxide (53.0 g, 0.48 mol), potassium dihydrogenophosphonate (13.60 g, 0.10 mol) in 1,4-dioxane (330 mL), was heated for 20 h (oil bath, 90°C), allowed to cool to room temperature and poured into water (750 mL). The aqueous solution was extracted with dichloromethane (3 x 250 mL) and the combined organic layers were washed with saturated aqueous sodium bicarbonate (1 x 50 mL), dried ($MgSO_4$), and evaporated. The residue was distilled under reduced pressure to afford pure **113** (V.P.C.) as a pale yellow oil (20.18 g, 41%): bp 97°C (5 mm) [lit.[7] 25%, bp 91--92°C (4 mm), lit.[8] 50%]; FT-IR (neat) 1646, 1634, 1578, 1520, 1471, 1252, 1214, 896, 833, 782, 578, 492 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 6.90--6.97 (m, 4 H), 7.03--

7.12 (m ,2 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 134.88, 136.22, 142.23, 188.07.

3,5-Cycloheptadienol (114).[9]

Sodium borohydride (4.00 g, 0.106 mol) was added portionwise to a magnetically stirred and cooled (ice bath) solution of tropone **113** (6.00 g, 0.056 mol) in a mixture of methanol (130 mL) and distilled water (20 mL), at such a rate that the temperature did not exceed 25°C. The mixture was stirred at room temperature for 2 h, glacial acetic acid (20 mL) was added and the stirring was continued for 5 min. Sodium bicarbonate was added until a neutral solution was obtained (pH paper) and the mixture was extracted with dichloromethane (4 x 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated, and the residue was distilled under reduced pressure to afford pure (V.P.C.) **114** as colorless oil (4.22 g, 68%): bp 73--74°C (7.5 mm) [lit.[9] 65%, 50--55°C (4 mm)]; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.467 (t, J = 5.0 Hz, 4 H), 4.11 (quintet, J = 5.0 Hz, 1 H), 5.55--5.85 (m, 4 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 39.30, 68.54, 126.12, 127.91.

(α,α,α)-6,7-Dioxabicyclo[3.2.2]non-8-en-3-ol acetate (116).[10]

Oxygen was passed through a stirred and cooled (0°C, immersion cooling bath, model Haake EK 51-1) solution of **114** (3.02, g, 27.4 mmol) and hematoporphyrin (3 mg) in methanol (75 mL). Two 300W bulbs were used as a source of light. The progress of the reaction was monitored by TLC (silica, ethyl acetate-dichloromethane 3:7), and, after one day, a considerable amount of starting material was still present. More hematoporphyrin (2 mg) was added and the reaction was continued for another day. The solvent was evaporated and the residue was taken up in dichloromethane (100 mL) and cooled to 0°C (ice bath). Pyridine (3.23 mL, 3.16 g, 40 mmol), followed by acetyl chloride (2.84 mL, 3,14 g, 40 mmol) was added at a fast dropwise rate to the stirred solution ,and the progress of the reaction was monitored by TLC (silica, ethyl acetate-dichloromethane 1:4). When all the alcohol had been acetylated (about 1 h), methanol (1 mL) was added and the stirring was continued for 0.5 h. The solution was washed with water (4 x 10 mL), dried ($MgSO_4$), and evaporated. The oily residue was dissolved in ether and crystallization was induced by cooling the solution (dry-ice cold bath). The solid was filtered off and recrystalized from ether to give pure (TLC) **116** as colorless crystals (2.74 g, 54%): mp 90.5-91.0 [lit.[10] 60%, 93.5--95°C]; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00 (s, 3 H), 2.02--2.41 (m , 2 H), 2.33--2.41 (m, 2 H), 4.69--4.81 (m 3 H), 6.41 (dd, J = 5.0, 3.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 21.15, 37.05, 68.02, 73.03, 128.65, 170.45.

(α,α,α)-6-Cycloheptaen-1,3,5-triol 3-acetate (117).

Zinc dust (500 mg) followed by glacial acetic acid (120 mg) was added to a stirred and cooled (0°C, ice bath) solution of peroxide **116** (201 mg, 1.09 mmol) in dichloromethane (10 mL). The reaction was monitored by TLC (silica, ethyl acetate--dichloromethane 3:7) and all starting material was consumed after 0.5 h. The reaction mixture was passed through a short silica pad (4 x 2 cm) and the product was eluted with ethyl acetate. The solvent was evaporated to give pure (TLC) **117** as colorless oil which solidified on standing (200 mg, 99%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66 (q, J = 11.5 Hz), 2.02 (s, 3 H), 2.05 (bdt, J = 11.5, 3 Hz, 2 H), 4.26 (bd, J = 11.0 Hz, 2 H), 4.93 (tt, J = 11.3 Hz. 3.5 Hz, 1 H), 5.64 (s, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 21.27, 41.83, 66.57, 69.90, 134.68, 170.90.

(*α,α,α*)-3,6-Bis(*tert*-butyldiphenylsilyloxy) cyclohepta-4-enol acetate(118).
tert-Butyldiphenylsilyl chloride (0.66 mL, 700 mg, 2.53 mmol) followed by imidazole (140 mg, 2.00 mmol) was added to a stirred solution of diol **117** (155 mg, 0.83 mmol) in a mixture of THF and dichloromethane (1:1, 15 mL). The solution was stirred for 5 h and evaporated. Flash chromatography of the residue over silica gel (20 x 1.5 cm) using dichloromethane gave (TLC) **118** (560 mg, 100%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.03 (s, 18 H), 1.68 (q, J = 11.3 Hz), 1.88--1.95 (m, 5 H), 4.09 (d, J = 10.5 Hz, 2 H), 4.45 (tt, J = 11.6 Hz, 3.6 Hz, 1 H), 5.64 (s, 1 H), 7.30--7.61 (m, 20 H) ; ^{13}C NMR ($CDCl_3$,

100.6 MHz) δ 19.08, 21.17, 26.90, 41.68, 68.00, 69.66, 127.62, 129.63, 133.77, 134.97, 135.64, 135.70, 169.38.

The following are exploratory experiments and full characterization of the products was not attempted.

(α,α,α)-3,6-Bis(tert-butyldiphenylsilyloxy) cyclohepta-4-enol (123).

A solution of sodium methoxide in methanol (1.0 M, 1.0 mL) was added to a stirred solution of **118** (310.0 mg, 0.467 mmol) in ether (10 mL). The progress of the reaction was monitored by TLC (silica, dichloromethane) and when all starting material was consumed (30 min), a solution of formic acid in ether (1.1 M, 0.9 mL) was added, and the resulting mixture was passed through a pad of silica (2 x 4 cm) and eluted with ether (100 mL). The solvent was evaporated and the resulting crude **123** (286.4 mg, 99%) was used without further purification: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.10 (s, 18 H), 1.73--1.83 (m, 2H), 1.85--1.98 (m, 3 H), 3.23 (bs, 1H), 4.11 (db, J = 10 Hz, 2 H), 5.67 (s, 1 H), 7.35[illegible]7.69 (m, 20 H).

(α,α,α)-1,5-Bis(tert-butyldiphenylsilyloxy)-3-methoxymethylcyclohepta-6-ene (124).

Compound **123** (1.220 g, 1.965 mmol) followed by methoxymethyl chloride (3.00 mL, 3.18 g, 39.6 mmol) was added to a suspension of sodium hydride [200 mg, 60%w/w oil suspension, 5.00 mmol, washed with dry THF (3 x 3 mL)] in dry THF (20 mL). The reaction mixture was refluxed under argon for 24 h, passed through a short pad of silica (2 x 4 cm) and eluted with ether (100 mL). Evaporation of the solvent and flash chromatography of the residue over silica (2.5 x 20 cm) with dichloromethane gave **124** as a colorless oil (587 mg, 45 %): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (s, 18 H), 1.49--1.60 (m, 2 H), 1.83--1.90 (m, 2 H), 3.00 (tt, J=11.0, 3.4 Hz, 1 H), 3.08 (s, 3 H), 4.05 (bd, J = 11 Hz, 2 H), 4.21 (s, 2 H), 5.78 (s, 2 H), 7.32--7.76 (m, 20 H).

(*syn, syn*)-2,6-Bis(*tert*-butyldiphenylsilyloxy)-4-methoxymethylhepta-1,7-diol (125).

Ozone was passed through a cooled (dry-ice bath) and stirred solution of **124** (587 mg, 0.883 mmol) in dichloromethane (10 mL) for 30 min. The solution turned pale blue. Next, argon was passed in for 10 min, and a solution of sodium borohydride (250 mg, 7.0 mmol) in methanol (3.0 mL) was then added. The dry-ice bath was replaced with an ice-bath and the mixture was stirred for 2 h. Acetic acid was added dropwise until no evolution of hydrogen was observed and the mixture was separated by flash chromatography over silica (2 x 20 cm) with 1:9 ethyl acetate--dichloromethane. Examination of the major component by TLC showed considerable tailing and partial decomposition. Sodium borohydride (500 mg, 14.0 mmol) was added to the combined fractions containing this material followed by methanol (3.0 mL), and the mixture was stirred overnight. After filtering the mixture through a silica pad (2 x 4 cm) and eluting with ethyl acetate (100 mL) the solvent was evaporated and the residue was separated by flash chromatography over silica (2 x 20 cm) 1:19 with ethyl acetate--dichloromethane to give pure (^{1}H NMR) **125** as colorless oil (185 mg, 30%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.10 (s, 18 H), 1.74 (t, J = 6.2 Hz, 4 H), 2.86 (d, J = 3.0 Hz, 3 H), 3.30 (s, 3 H), 3.55--3.67 (m, 4 H), 3.85--3.94 (m, 2 H), 3.98 (q, J = 6.2 Hz, 1 H), 4.60 (s, 2 H), 7.35--7.71 (m, 20 H).

(*syn*, *syn*)-2,6-Bis(*tert*-butyldiphenylsilyloxy)-4-acetoxyheptadial (126).

Ozone was passed through a cooled (dry-ice bath) and stirred solution of **118** (710 mg, 1.070 mmol) in dichloromethane (18 mL) for 30 min. The solution turned pale blue. Next, argon was passed in for 10 min and triphenylphosphine (842 mg, 3.21 mmol) was added. The cold bath was removed. The mixture was stirred at room temperature for 1 h. Flash chromatography over silica (2.5 x 20 cm) with dichloromethane gave **126** as colorless oil (672 mg, 90%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (s, 18 H), 1.79 (s, 3 H), 1.80--1.93 (m ,4 H), 4.04 (dd, J = 7.5, 3.0 Hz, 2 H), 5.40 (tt, J = 9.0, 4.3 Hz, 1 H), 7.32--7.73 (m, 20 H).

(*syn*, *syn*)-2,6-Bis(*tert*-butyldiphenylsilyloxy)-4-acetoxyhepta-1,7-diol (127).

Acetic acid (200 mg, 3.32 mmol) followed by a solution of sodium cyanoborohydride (150 mg, 2.38 mmol) in methanol (2.0 mL) was added to a stirred solution of dialdehyde **126** (545 mg, 0.784 mmol) in ether (20 mL). The mixture was stirred for 2 h, filtered thorough a silica pad (2 x 6 cm) with ether and evaporated to give **127** (538 mg, 98%). This was used without further purification and had: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.04 (s, 18 H), 1.44--1.52 (m, 2 H), 1.61 (s, 3 H), 1.73--1.81 (m ,2 H), 1.91 (bs, 2 H), 3.35--3.52 (m, 4 H), 3.61--3.68 (m, 2 H), 4.81 (tt, J = 8.9, 4.2 Hz, 1 H), 7.32--7.64 (m, 20 H).

(*syn*, *syn*)-2,6-Bis(*tert*-butyldiphenylsilyloxy)-4-acetoxyhepta-1,7-diol dimethanesulfonate (128). Methanesulfonyl chloride (0.750 mL, 0.977 g, 9.46 mmol) followed by imidazole (600 mg, 8.81 mmol) was added to a solution of diol **127** (517 mg, 0.740 mmol) in dichloromethane (12 mL). The mixture was stirred for 3 days. Flash chromatography of the solution over silica (2.5 x 20 cm) with ethyl acetate--dichloromethane (1:9) gave **128** as a colorless oil (420 mg, 66%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (s, 18 H), 1.50--1.82 [m, 3 H (including singlet at 1.63)], 2.84 (s, 6 H), 3.72--3.86 (m, 2 H), 2.94--3.16 (m, 4 H), 4.89 (tt, J = 8.9, 6,2 Hz, 1 H), 7.32--7.76 (m, 20 H).

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APPENDIX B

Biological assay.

Solutions (or suspensions) of the materials to be tested and a solution of Amphotericin B, in DMSO, all of concentration 4.0 mg/mL, were prepared by dissolving samples (about 1 mg) in the appropriate amount of solvent.

The nutrient medium consisted of a solution of a commercial yeast extract (2%) and glucose (1%) in water and the pH was adjusted to 4.5 with hydrochloric acid.

For each tested substance and for Amphotericin B, a set of ten test tubes was prepared. The first test tube contained 3.80 mL of the medium and each of the remaining test tubes contained 2.00 mL of the medium. A previously prepared solution of the test substance (0.200 mL) was added to the first test tube, the sample was thoroughly mixed and a 2.00 mL portion was withdrawn and transferred to the second test tube. This operation of dilution was carried out until the ninth test tube was reached. A sample (2.00 mL) of the solution was withdrawned from this test tube and discarded. The test tube no. 10 was used as a blank.

Next, all test tubes were inoculated with 50 µL of the yeast cell suspension (Sacharomyces Cerevisiae SK1, 9 x 10^6 cells/mL), incubated for 24 h at 27°C, inspected, incubated for another 24 h at 27°C and inspected again.

The amount of growth was determined visually by the amount of sediment and assigned a value from 0 to 3+ (i.e. 0, +, 1, 1+ ...).

There was no growth (0) in AmB inhibited test tubes 1--7 and some growth in test tubes 8 (+) and 9 (2). In the case of all tested analogues a considerable growth (2 to 3+) was observed in the test tube no. 1 (which contained the highest concentration of test compound).

APPENDIX C

This appendix contains the X-ray data for compound **102**, this is unedited original report.

SR:rgb0089b 03-Aug-89

MERCK SHARP & DOHME RESEARCH LABORATORIES

Biophysical Chemistry

Structure Determination Laboratory

Report[1] on the
Complete Refinement of
$C_{35}H_{52}O_4S_4$
for
D.L.J. Clive
University of Alberta

EXPERIMENTAL

Data Collection

A red, needle-shaped crystal of $C_{35}H_{32}O_4S_4$, having approximate dimensions of 0.14 x 0.07 x 0.53 mm, was mounted in a non-specific orientation on an Enraf-Nonius CAD4 automated diffractometer. All intensity measurements were performed using Cu K_α radiation ($\lambda = 1.54184$ Å) with a graphite crystal, incident beam monochromator.

The automatic peak search and reflection indexing programs[2] in conjunction with a cell reduction program showed the crystal to be monoclinic and from the systematic absences of:

$$h0l, h+l \text{ odd}$$

$$0k0, k \text{ odd}$$

the space group was determined to be $P2_1/n$, an alternative setting of $P2_1/c$ (No. 14)[3].

Cell constants were obtained from a least-squares refinement of the setting angles of 25 reflections in the range $14 < 2\theta < 46°$. The various crystal parameters are given in Table 1.

The intensity data were collected at room temperature (23° C) using an ω scan ranging in speed from 10.1 to $3.3° \text{min}^{-1}$. The variable scan rate was chosen to give $\sigma(I)/I \leq 0.05$ within a time limit of 30 s in order to achieve improved counting statistics for both intense and weak reflections in a minimum time. The scan range was determined as a function of θ to compensate for the $\alpha_1 - \alpha_2$ wave-length dispersion:

$$\text{omega scan width} = 0.80 + 0.14\tan(\theta)°$$

Backgrounds for the peaks were measured by extending the scan 25% on either side of the calculated range to give a peak to background counting time of 2:1. Intensity measurements were made out to a maximum 2θ of 130°. There were 2 reflections which were were chosen as standard reflections and these were remeasured every 60 min of exposure time to check on crystal and electronic stability over the course of data collection. These reflections changed in intensity by -9.5% and -11.1%, respectively, over the time span of data collection. A linear decay correction was applied to the data.

Data Reduction

A total of 7201 reflections were collected and these were corrected for Lorentz, polarization and background effects according to the following formulæ:

$$I = SR(SC - R * B)/Lp$$

$$\sigma^2(I) = SR(SC + R^2B) + (pI)/Lp$$

where SR is the scan rate, SC is the total scan count, R is the ratio of scan time to background time, B is the total background count, p is a factor to down weight intense reflections (chosen as 0.04 in this experiment) and Lp is the Lorentz and polarization correction term. After averaging equivalent forms (R-Factor for averaging is 0.017) and rejecting any systematically absent data there were 6513 unique reflections of which 2080, having $I > 3\sigma(I)$, were used in the structure solution and refinement.

Structure Solution and Refinement

The structure was solved using the direct methods program SHELXS-86[4] which gave the positional parameters for the some of the atoms. During the subsequent stages of least-squares refinement and difference Fourier synthesis it became apparent that a large portion of the molecule which included the sulphur-carbon chain was disordered. Eventually a connected chain was determined along with several atoms at their disordered positions. However, this model only partially describes the true disorder and the refinement of the model has given only a marginally satisfactory result.

Refinement[5] of atomic parameters was carried out by using full-matrix least-squares techniques on F_o minimizing the function

$$\Sigma \mathrm{w}(|F_o| - |F_c|)^2$$

where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes respectively, and the weighting factor w is given by

$$\mathrm{w} = 4F_o^2/\sigma(F_o)^2$$

The neutral atom scattering factors were calculated from the analytical expression for the scattering factor curves[6]. The f' and f'' components of anomalous dispersion[7] were included in the calculations for all non-hydrogen atoms.

Prior to the final cycles of refinement several of the atoms involved in the disordered chain had their parameters fixed as these did not tend to converge to stable values.

In the final cycle 230 parameters were refined using 2080 observations having $I >$

$3\sigma(I)$. The final agreement factors were

$$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.141, \text{and}$$

$$R_2 = (\Sigma \mathrm{w}(|F_o| - |F_c|)^2/\Sigma \mathrm{w} F_o^2)^{1/2} = 0.183$$

The largest shift in any parameter was 0.04 times its estimated standard deviation and the error in an observation of unit weight was 4.98e. An analysis of R_2 in terms of F_o, $\lambda^{-1}\sin\theta$, and various combinations of Miller indices showed no unusual trends. The highest peak in the final difference Fourier has a density of 0.8(1) eÅ^{-3} and is located within the chain of disordered atoms approximately 1.5 Å from S2.

References and Notes

1. This X-ray crystallographic study was carried out by Dr. R.G. Ball, Biophysical Chemistry Department, RY80M-203, Merck Sharp & Dohme Research Laboratories, PO Box 2000, Rahway, New Jersey, USA 07065. Inquiries regarding the crystallographic results should be directed to the above address quoting report number SR:rgb0089b.

2. The diffractometer programs are those supplied by Enraf-Nonius for operating the CAD4F diffractometer with some local modifications and additions.

3. *International Tables for X-Ray Crystallography* (1969). Vol. I. Birmingham: Kynoch Press.

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5. The computer programs used in this analysis include the Enraf-Nonius *Structure Determination Package*. *Version* 3 (1985, Delft, The Netherlands) rewritten for a Sun Microsystems computer and several locally written or modified programs.

6. *International Tables for X-Ray Crystallography* (1974). Vol. IV. Table 2.2B. Birmingham: Kynoch Press. (Present distributor D. Reidel, Dordrecht.)

7. *ibid*., Table 2.3.1.

Table 1. Experimental Details

A. Crystal Data

$C_{35}H_{52}O_4S_4$; FW = 665.06

Crystal dimensions: 0.14 x 0.07 x 0.53 mm

monoclinic space group $P2_1/n$

$a = 6.340\,(6)$, $b = 23.89\,(1)$, $c = 24.85\,(1)$ Å

$\beta = 96.43\,(8)^\circ$

$V = 3740$ Å^3; $Z = 4$; $D_c = 1.181$ g cm^{-3}; $\mu = 25.52$ cm^{-1}

B. Data Collection and Refinement Conditions

Radiation:	Cu K_α ($\lambda = 1.54184$ Å)
Monochromator:	incident beam, graphite crystal
Take-off angle:	3.5°
Detector aperture:	2.40 mm horiz x 4.0 mm vert
Crystal-to-detector distance:	205 mm
Scan type:	ω
Scan rate:	10.1 – 3.3°min^{-1}
Scan width:	$0.80 + 0.14\tan(\theta)^\circ$
Data collection 2θ limit:	130°
Data collection index range:	$+h, +k, \pm l$
Reflections measured:	6513 unique, 2080 with $I > 3\sigma(I)$
Observations:variables ratio:	2080: 230
Agreement factors R_1, R_2, GOF:	0.141, 0.183, 4.98
Corrections applied:	Linear decay correction

Table of Positional and Thermal Parameters and Their Estimated Standard Deviations.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S1	93.9(8)	313.3(2)	704.0(2)	13.0(4)	13.3(4)	16.2(4)	1.0(3)	-3.7(3)	-3.9(3)
S2	682(1)	240.8(4)	602.9(3)	18.3(6)	26.8(8)	22.3(7)	0.5(6)	-0.2(6)	7.3(6)
S3	1116(1)	304.8(3)	474.4(2)	17.1(5)	26.0(7)	14.6(5)	4.5(5)	0.3(4)	0.0(5)
S4	1449.9(9)	341.9(2)	309.0(2)	14.7(4)	11.9(4)	16.0(4)	0.1(3)	-3.9(3)	1.4(3)
O1	459(2)	381.3(5)	918.6(3)	12.0(8)	21(1)	7.0(6)	-2.3(8)	3.1(6)	2.4(7)
O2	773(2)	393.6(5)	887.5(3)	9.9(7)	23(1)	6.1(6)	-3.5(7)	0.3(5)	2.0(7)
O3	998(2)	453.7(5)	118.1(3)	11.1(7)	20(1)	5.0(5)	-1.0(7)	3.5(5)	1.3(6)
O4	687(2)	460.0(5)	150.3(3)	10.1(7)	17(1)	6.5(6)	-1.1(7)	0.1(5)	0.2(6)
C31	886(3)	377.6(9)	946.9(7)	17(2)	17(2)	9(1)	-1(2)	0(1)	0(1)
C32	921(3)	432.6(8)	968.7(7)	21(2)	16(2)	11(1)	-5(1)	3(1)	0(1)
C33	903(2)	454.6(7)	156.4(5)	10(1)	12(1)	6.2(8)	-2(1)	0.3(8)	0.8(9)
C34	572(3)	466.3(8)	94.2(5)	15(1)	15(1)	5.2(8)	-1(1)	-1.1(9)	-1(1)
C35	528(3)	410.8(8)	73.7(6)	14(1)	18(2)	6.8(9)	-5(1)	2.5(9)	-2(1)

Atom	x	y	z	U	Atom	x	y	z	U
C1	468(2)	399.3(5)	824.0(5)	7.1(4)	C14	1442(4)	349(1)	232.5(9)	6.5(7)
C2	231(2)	392.2(6)	814.7(5)	8.4(4)	C14'	1225(7)	337(2)	243(2)	19(2)
C3	174(2)	329.3(6)	814.2(6)	10.0(5)	C15	1250(3)	375.6(7)	207.8(6)	11.9(6)
C4	239(4)	294(1)	780(1)	8.6(9)	C16	1226(2)	437.4(6)	223.1(5)	7.6(4)
C4'	264(4)	297(1)	760(1)	8.7(9)	C17	993(2)	450.6(5)	213.4(5)	7.1(4)
C5	279(5)	300(1)	647(1)	11(1)	C18	862(2)	460.4(5)	253.5(5)	7.6(4)

Table of Positional and Thermal Parameters and Their Estimated Standard Deviations (continued).

Atom	x	y	z	U	Atom	x	y	z	U
C6	299	235	644	10	C19	943(2)	456.4(5)	311.5(5)	7.7(4)
C6'	312	257	655	22	C20	795(2)	458.8(5)	347.8(5)	7.4(4)
C7	430(3)	217(1)	592.3(9)	17.9(9)	C21	870(2)	453.3(5)	406.8(5)	7.1(4)
C8	740	221	523	13	C22	727(2)	447.8(6)	442.9(5)	8.3(4)
C8'	735	285	542	23	C23	798(2)	443.9(6)	502.0(5)	8.9(4)
C9'	793	282	496	41	C24	657(2)	440.1(5)	539.6(5)	7.7(4)
C9	704	257	502	9	C25	727(2)	435.6(5)	596.3(5)	7.4(4)
C10'	904	239	497	30	C26	595(2)	432.3(5)	635.0(5)	6.8(4)
C10	852	286	456	20	C27	663(2)	426.7(5)	691.3(5)	7.3(4)
C11	1139(4)	322(1)	404(1)	23(1)	C28	523(2)	420.1(5)	728.6(5)	7.3(4)
C12	1236(4)	274(1)	375.3(9)	21(1)	C29	598(2)	413.5(5)	785.9(5)	6.9(4)
C13	1240(4)	291(1)	318(1)	20(1)	C30	560(2)	390.4(6)	881.1(5)	8.9(5)

The atomic positional parameters have been multiplied by 10^3.
The anisotropic thermal parameters have been multiplied by 10^2.
The isotropic thermal parameters have been multiplied by 10^2.
The form of the anisotropic thermal parameter is $\exp[-2\pi^2(h^2a^{*2}U_{11}+k^2b^{*2}U_{22}+l^2c^{*2}U_{33}+2hka^*b^*U_{12}+2hla^*c^*U_{13}+2klb^*c^*U_{23})]$
Estimated standard deviations in the least significant digits are shown in parentheses. Those parameters without an error were not refined.

Table of Anisotropic and Equivalent Isotropic Thermal Parameters

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}	B_{equiv}
S1	13.0(4)	13.3(4)	16.2(4)	1.0(3)	-3.7(3)	-3.9(3)	11.5(2)
S2	18.3(6)	26.8(8)	22.3(7)	0.5(6)	-0.2(6)	7.3(6)	17.9(3)
S3	17.1(5)	26.0(7)	14.6(5)	4.5(5)	0.3(4)	0.0(5)	15.3(3)
S4	14.7(4)	11.9(4)	16.0(4)	0.1(3)	-3.9(3)	1.4(3)	11.5(2)
O1	12.0(8)	21(1)	7.0(6)	-2.3(8)	3.1(6)	2.4(7)	10.4(4)
O2	9.9(7)	23(1)	6.1(6)	-3.5(7)	0.3(5)	2.0(7)	10.2(4)
O3	11.1(7)	20(1)	5.0(5)	-1.0(7)	3.5(5)	1.3(6)	9.3(4)
O4	10.1(7)	17(1)	6.5(6)	-1.1(7)	0.1(5)	0.2(6)	8.9(4)
C31	17(2)	17(2)	9(1)	-1(2)	0(1)	0(1)	11.6(7)
C32	21(2)	16(2)	11(1)	-5(1)	3(1)	0(1)	12.5(8)
C33	10(1)	12(1)	6.2(8)	-2(1)	0.3(8)	-0.8(9)	7.5(5)
C34	15(1)	15(1)	5.2(8)	-1(1)	-1.1(9)	-1(1)	9.5(6)
C35	14(1)	18(2)	6.8(9)	-5(1)	2.5(9)	-2(1)	10.1(6)

The anisotropic thermal parameters have been multiplied by 10^2.
The form of the anisotropic thermal parameter is:
$\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})]$
Estimated standard deviations in the least significant digits are shown in parentheses.

Table of Positional (x 10^3) and Thermal (x 10^2) Parameters

Atom	x	y	z	U, Å^2	Atom	x	y	z	U, Å^2
S1	93.9(8)	313.3(2)	704.0(2)	14.5(2)*	C13	1240(4)	291(1)	318(1)	20(1)
S2	682(1)	240.8(4)	602.9(3)	22.7(4)*	C14	1442(4)	349(1)	232.5(9)	8.5(7)
S3	1116(1)	304.8(3)	474.4(2)	19.4(3)*	C14'	1225(7)	337(2)	243(2)	19(2)
S4	1449.9(9)	341.9(2)	309.0(2)	14.8(2)*	C15	1250(3)	375.6(7)	207.8(6)	11.9(6)
O1	459(2)	381.3(5)	918.6(3)	13.2(5)*	C16	1226(2)	437.4(6)	223.1(5)	7.6(4)
O2	773(2)	393.6(5)	887.5(3)	12.9(5)*	C17	993(2)	450.6(5)	213.4(5)	7.1(4)
O3	998(2)	453.7(5)	118.1(3)	11.8(5)*	C18	862(2)	460.4(5)	253.5(5)	7.6(4)
O4	687(2)	460.0(5)	150.3(3)	11.3(4)*	C19	943(2)	456.4(6)	311.5(5)	7.7(4)
C1	468(2)	399.3(5)	824.0(5)	7.1(4)	C20	795(2)	458.8(5)	347.8(5)	7.4(4)
C2	231(2)	392.2(6)	814.7(5)	8.4(4)	C21	870(2)	453.3(5)	406.8(5)	7.1(4)
C3	174(2)	329.3(6)	814.2(6)	10.0(5)	C22	727(2)	447.8(6)	442.9(5)	8.3(4)
C4	239(4)	294(1)	780(1)	8.6(9)	C23	798(2)	443.9(6)	502.0(5)	8.9(4)
C4'	264(4)	297(1)	760(1)	8.7(9)	C24	657(2)	440.1(5)	539.6(5)	7.7(4)
C5	279(5)	300(1)	647(1)	11(1)	C25	727(2)	435.6(5)	596.3(5)	7.4(4)
C6	299	235	644	10	C26	595(2)	432.3(5)	635.0(5)	6.8(4)
C6'	312	257	655	22	C27	663(2)	426.7(5)	691.3(5)	7.3(4)
C7	430(3)	217(1)	592.3(9)	17.9(9)	C28	523(2)	420.1(5)	728.6(5)	7.3(4)
C8	740	221	523	13	C29	598(2)	413.5(5)	785.9(5)	6.9(4)
C8'	735	285	542	23	C30	560(2)	390.4(6)	881.1(5)	8.9(5)
C9'	793	282	496	41	C31	886(3)	377.6(9)	946.9(7)	14.7(9)*
C9	704	257	502	9	C32	921(3)	432.6(8)	968.7(7)	16(1)*
C10'	904	239	497	30	C33	903(2)	454.6(7)	156.4(5)	9.4(6)*

Table of Positional (x 10^3) and Thermal (x 10^2) Parameters (continued)

Atom	x	y	z	U, Å^2	Atom	x	y	z	U, Å^2
C10	852	286	456	20	C34	572(3)	466.3(8)	94.2(5)	12.0(7)*
C11	1139(4)	322(1)	404(1)	23(1)	C35	528(3)	410.8(8)	73.7(6)	12.7(8)*
C12	1236(4)	274(1)	375.3(9)	21(1)					

*Indicates an atom refined anisotropically.

The equivalent isotropic thermal parameter U is given by $U = 1/3\sum_{i=1}^{3} r_i^2$, where r_i are the root-mean-square amplitudes of vibration.

Those parameters without an esd were not refined.

Table of Root-Mean-Square Amplitudes of Thermal Vibration in Angstroms.

Atom	min	int'med	max	Atom	min	int'med	max
S1	0.299	0.349	0.475	O4	0.253	0.319	0.416
S2	0.395	0.446	0.569	C31	0.306	0.406	0.425
S3	0.371	0.404	0.529	C32	0.316	0.370	0.489
S4	0.312	0.350	0.467	C33	0.246	0.297	0.367
O1	0.231	0.351	0.469	C34	0.217	0.382	0.409
O2	0.242	0.300	0.488	C35	0.252	0.323	0.463
O3	0.188	0.343	0.447				

Table of Selected Interatomic Distances[a]

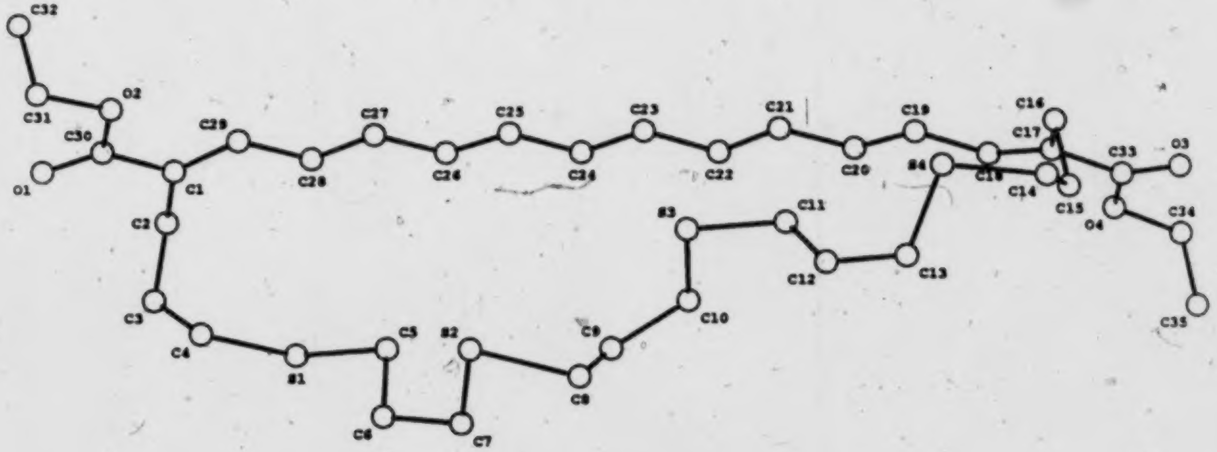
Atom1	Atom2	Distance	Atom1	Atom2	Distance	Atom1	Atom2	Distance
S1	C4	2.06 (2)	C1	C30	1.487 (9)	C14	C15	1.44 (1)
S1	C4'	1.70 (2)	C2	C3	1.545 (9)	C14'	C15	1.31 (2)
S1	C5	1.97 (1)	C3	C4	1.30 (2)	C15	C16	1.54 (1)
S2	C7	1.69 (1)	C3	C4'	1.72 (2)	C16	C17	1.504 (8)
S2	C8	2.099 (4)	C5	C6	1.55 (2)	C17	C18	1.388 (8)
S2	C8'	1.906 (4)	C6	C7	1.67 (1)	C17	C33	1.470 (6)
S3	C10	1.744 (4)	C8	C8'	1.5794 (8)	C18	C19	1.479 (8)
S3	C11	1.81 (1)	C8	C9'	1.6554 (8)	C19	C20	1.372 (7)
S4	C13	1.84 (1)	C8	C10'	1.354 (1)	C20	C21	1.496 (8)
S4	C14	1.91 (1)	C8'	C9'	1.2308 (7)	C21	C22	1.350 (7)
S4	C14'	2.05 (2)	C8'	C9	1.19	C22	C23	1.491 (8)
O1	C30	1.210 (8)	C9'	C10'	1.2474 (6)	C23	C24	1.367 (8)
O2	C30	1.345 (8)	C9'	C10	1.1052 (6)	C24	C25	1.431 (8)
O2	C31	1.614 (9)	C9	C10'	1.353 (1)	C25	C26	1.346 (7)
O3	C33	1.184 (8)	C9	C10	1.695 (1)	C26	C27	1.426 (7)
O4	C33	1.366 (8)	C10'	C10	1.5269 (6)	C27	C28	1.362 (7)
O4	C34	1.508 (8)	C11	C12	1.51 (2)	C28	C29	1.460 (8)
C1	C2	1.504 (8)	C12	C13	1.48 (1)	C31	C32	1.43 (1)
C1	C29	1.367 (7)	C14	C14'	1.46 (2)	C34	C35	1.44 (1)

[a] In angstroms. Numbers in parentheses are estimated standard deviations in the least significant digits.

Table of Selected Interatomic Angles[a]

Atom1	Atom2	Atom3	Angle
C4	S1	C5	112.5 (7)
C4'	S1	C5	100.0 (7)
C7	S2	C8	92.3 (4)
C7	S2	C8'	108.0 (4)
C13	S4	C14	104.2 (6)
C30	O2	C31	115.5 (6)
C33	O4	C34	119.1 (6)
C2	C1	C29	126.3 (6)
C2	C1	C30	114.3 (6)
C29	C1	C30	119.3 (6)
C1	C2	C3	109.9 (5)
C2	C3	C4	123 (1)
C2	C3	C4'	110.3 (7)
S1	C4	C3	108 (1)
S1	C4'	C3	108 (1)
S1	C4'	C4	124 (4)
S1	C5	C6	104.7 (8)
S1	C5	C6'	98.1 (9)
C5	C6	C7	110.0 (7)
C6'	C6	C7	121.7 (4)
S2	C7	C6	109.7 (6)
S2	C8	C9'	104.8 (1)
S2	C8	C9	105.1 (1)
S2	C8	C10'	127.5 (1)
S2	C8'	C9	109.7 (1)
C8	C9'	C10	122.96 (2)
C8'	C9'	C10'	104.28 (4)
C9	C9'	C10	120.18 (4)
C8	C9	C8'	90.86 (4)
C8	C9	C9'	124.64 (5)
C8	C9	C10	125.93 (3)
C8'	C9	C10'	100.53 (7)
C8'	C9	C10	106.59 (4)
C8	C10'	C10	115.62 (4)
S3	C10	C9'	101.4 (1)
S3	C10	C9	121.0 (1)
S3	C11	C12	112 (1)
C11	C12	C13	108 (1)
S4	C13	C12	112.8 (9)
S4	C14	C15	113.1 (8)
S4	C14'	C15	112 (2)
C14	C15	C16	114.5 (7)
C14'	C15	C16	120 (1)
C15	C16	C17	106.6 (5)
C16	C17	C18	125.3 (6)
C16	C17	C33	116.1 (6)
C18	C17	C33	118.5 (7)
C17	C18	C19	121.2 (6)
C18	C19	C20	116.7 (6)
C19	C20	C21	118.4 (6)
C20	C21	C22	119.8 (6)
C21	C22	C23	120.7 (6)
C22	C23	C24	122.1 (6)
C23	C24	C25	121.5 (6)
C24	C25	C26	123.9 (6)
C25	C26	C27	124.1 (6)
C26	C27	C28	121.8 (6)
C27	C28	C29	120.7 (6)
C1	C29	C28	123.2 (6)
O1	C30	O2	122.1 (7)
O1	C30	C1	125.0 (8)
O2	C30	C1	112.9 (7)
O2	C31	C32	99.3 (8)
O3	C33	O4	120.5 (7)
O3	C33	C17	126.4 (8)
O4	C33	C17	113.1 (7)
O4	C34	C35	106.8 (7)

[a] In degrees. Numbers in parentheses are estimated standard deviations in the least significant digits.


C32
C31
O2
C30
O1
C1
C29
C2
C28
C27
C26
C25
C24
C23
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C21
C20
C19
C18
C17
C16
C14
C15
C33
O3
O4
C34
C35
C3
C4
S1
C5
C6
C7
S2
C8
C9
C10
S3
C11
C12
C13
S4

Figure 1. Perspective view of the molecule showing the atom numbering scheme for one possible chain of atoms. Atoms are drawn at an arbitrary size.

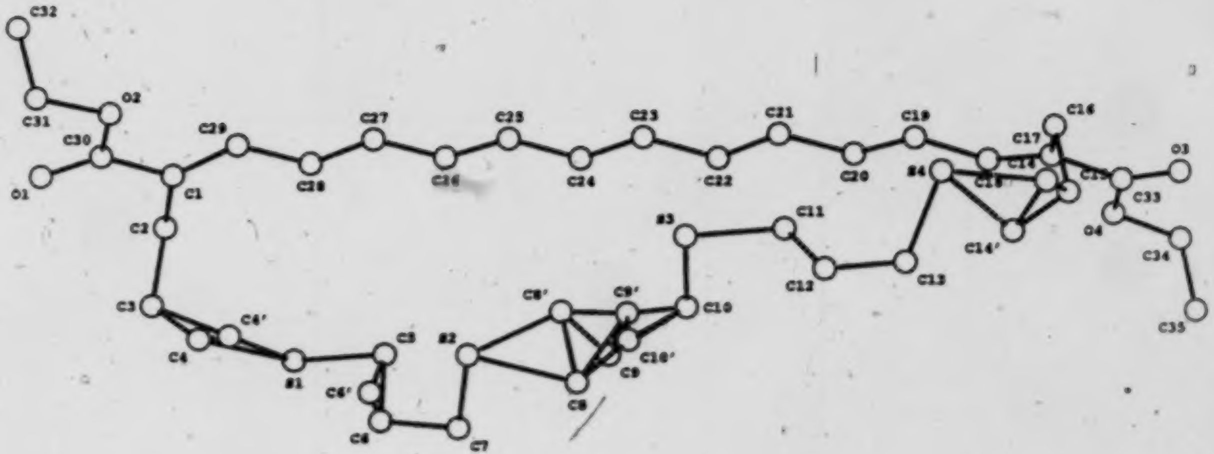
C32
O2
C31
C30
O1
C1
C29
C2
C28
C27
C26
C25
C24
C23
C22
C21
C20
C19
S4
C18
C17
C16
C14
C15
O3
C33
O4
C34
C35
C14'
S3
C11
C12
C13
C3
C4'
C4
S1
C5
C6'
C6
C7
S2
C8'
C9'
C10
C10'
C9
C8

Figure 2. View of the molecule showing the atoms included to compensate for the observed disorder.